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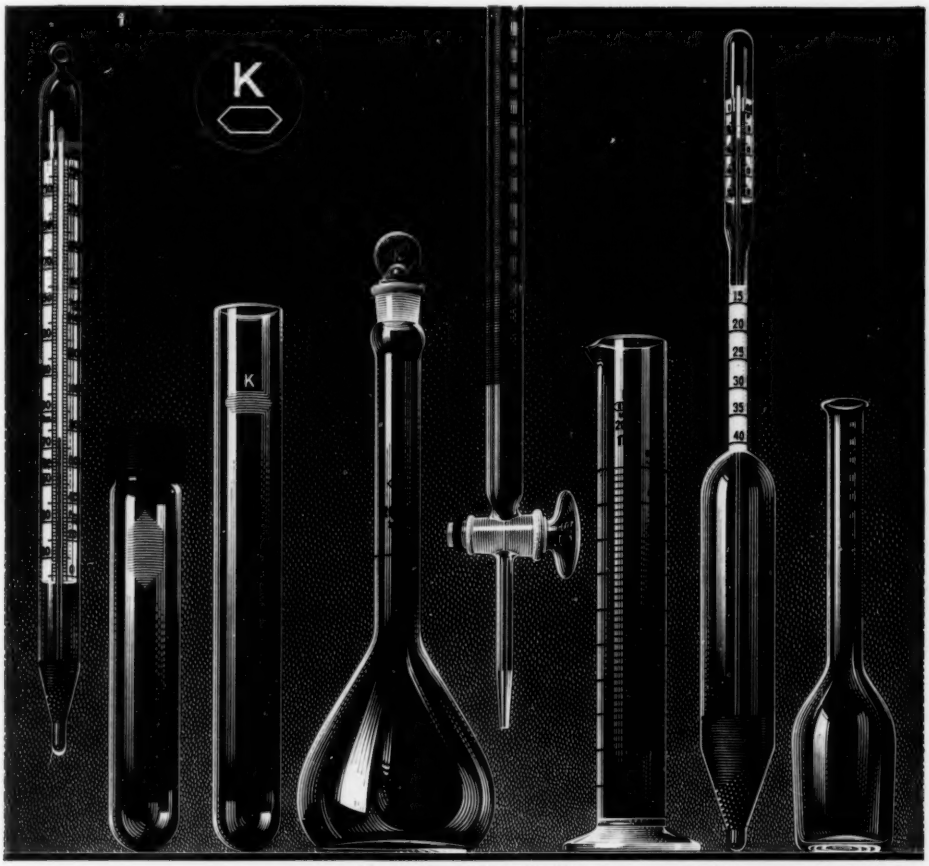

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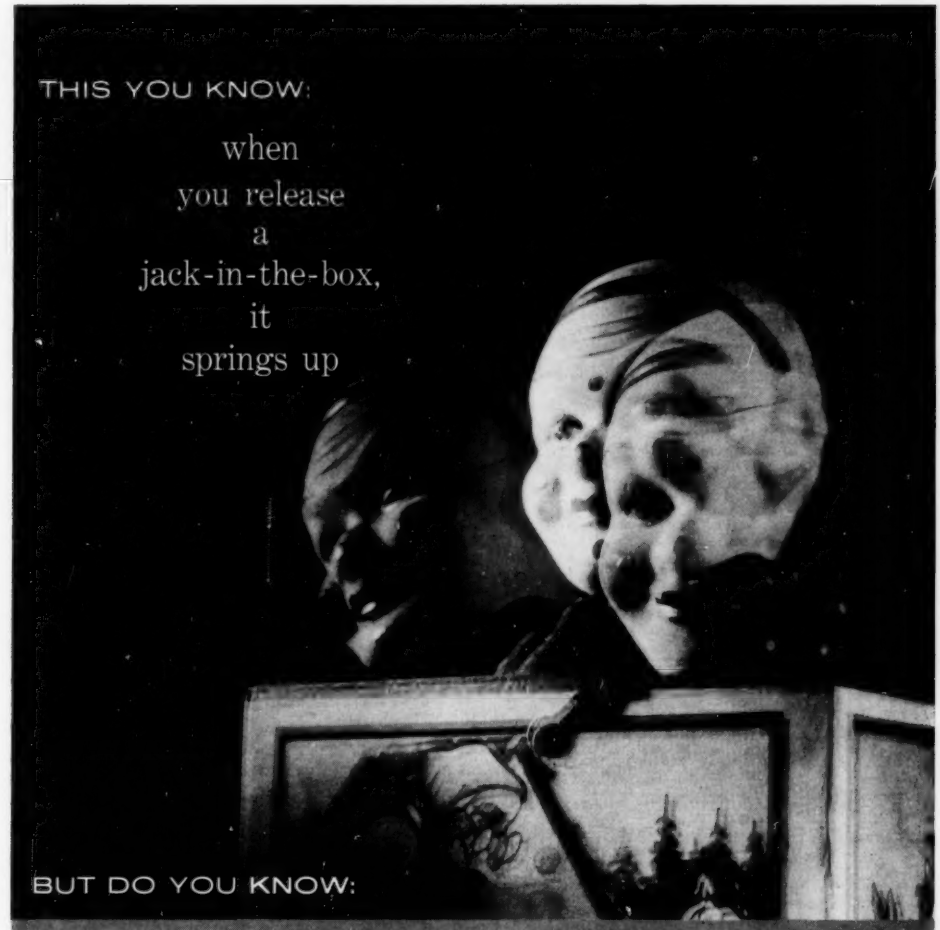

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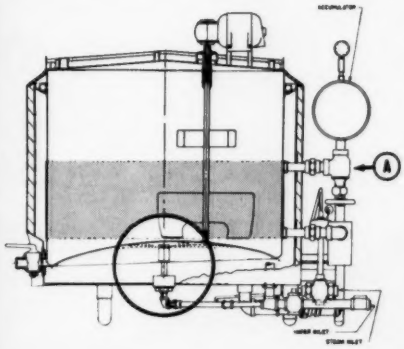

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STUDIES ON THE COLLOIDAL PROTEINS OF SKIMMILK. II. THE EFFECT OF HEAT AND DISODIUM PHOSPHATE ON THE COMPOSITION OF THE CASEIN COMPLEX[1]

L. F. EDMONDSON[2] AND N. P. TARASSUK

Department of Dairy Industry, University of California, Davis

Investigators agree that casein exists in milk as a calcium salt together with a quantity of additional calcium phosphate usually referred to as the "colloidal calcium phosphate." In addition to this complex, milk also contains calcium and phosphate ions in solution. The distinction between the colloidal and soluble phases is based on the separation of the former by such procedures as ultrafiltration, centrifugation, or rennet coagulation. The distinction between the two forms of calcium in the colloidal phase is based on the calcium-binding capacity of casein as a function of pH. The amount "bound" at the pH of normal milk, 6.7, has been shown to be slightly more than 10 mg. per gram of casein *(8, 13)*. The form of the colloidal calcium phosphate is uncertain. Van Slyke and Bosworth *(15)* believed it to be diphosphate ($CaHPO_4$). Most recent workers *(3, 10, 11, 14)*, however, have found a calcium-phosphorus ratio of about 1.50, which agrees with the formula for triphosphate, $Ca_3(PO_4)_2$.

The authors in a previous paper *(4)* discussed the effects that preheating treatments and adding disodium phosphate had on the centrifugal sedimentation rates of the calcium caseinate complex. The samples collected from the centrifugal runs also afforded a means of studying the effect of these treatments on the composition of the complex as it sedimented in the centrifuge. By analyzing the supernatant liquids for calcium and phosphorus in addition to nitrogen, changes in the calcium-nitrogen and phosphorus-nitrogen ratios of the sediment were determined.

EXPERIMENTAL PROCEDURE

The methods of heating the skimmilk, adding the disodium phosphate, and collecting the supernatant liquid from the centrifuged samples were the same as reported previously *(4)*.

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[1] The data are part of a thesis presented to the Graduate School of the University of California by the senior author in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1954.

[2] Present address: Eastern Utilization Research Branch, USDA, Washington, D. C.

Analyses were made on the original skimmilk and on the supernatant liquids from centrifuged milks. Nitrogen determinations were made by the Kjeldahl procedure. The noncasein nitrogen fraction was prepared for analyses by the Rowland procedure *(12)*. Total calcium was determined according to a modified form of the A.O.A.C. procedure *(1)* for calcium in cereal foods. Similarly, total phosphorus was determined according to the A.O.A.C. procedure for soils.

RESULTS AND DISCUSSION

The relationship between total calcium and total nitrogen in the supernatant liquids of samples centrifuged for successively increasing periods of time is shown in Figure 1. Similarly, the relationship between total phosphorus and total nitrogen is shown in Figure 2. For the calcium-nitrogen relationship the

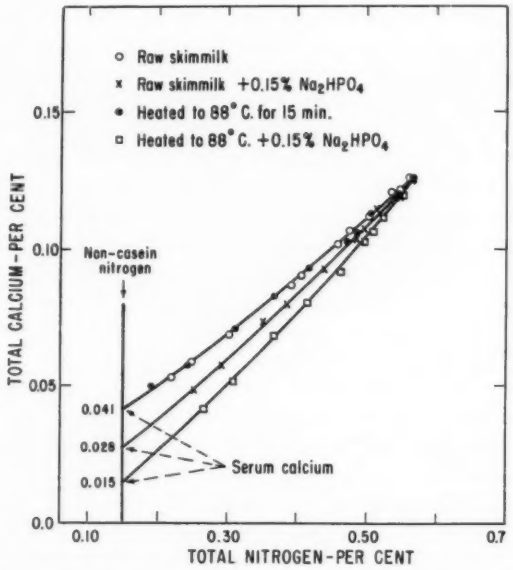

FIG. 1. The relationship between the total calcium and total nitrogen in the supernatant liquid from skimmilk centrifuged for progressively increasing periods of time.

lines for all four samples have a very slight curvature. The direction of curvature shows that a larger amount of calcium is associated with the first particles removed than with those removed only after prolonged centrifugation. Straight lines for these plots would indicate that the composition of the sediment, with respect to calcium and nitrogen, was constant regardless of particle size. More nearly straight lines were obtained for three other samples similarly studied, particularly for the raw milk samples. The latter results are in agreement with those of Ford and Ramsdell *(6)* and De Kadt and Van Minnen *(3)*. In any case, the variation from a straight-line relationship is only slight, so that for the

purpose of calculating the average composition of the complex a straight-line relationship is assumed.

Straight lines appear to best represent the phosphorus-nitrogen relationship, as shown in Figure 2. The lines representing this relationship do not diverge from a common uppermost point because of the phosphate added to two of the samples.

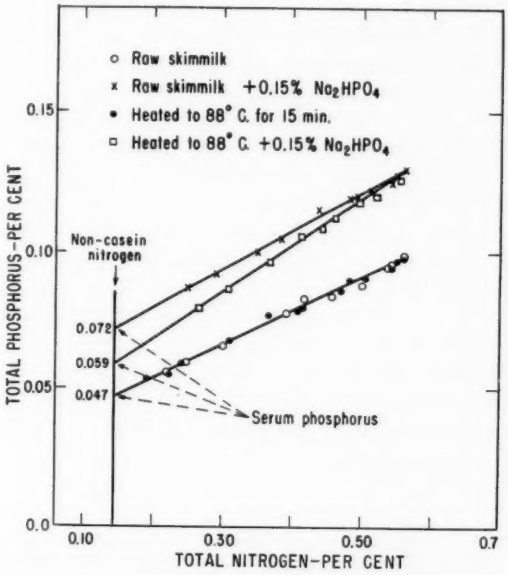

FIG. 2. The relationship between the total phosphorus and total nitrogen in the supernatant liquid from skimmilk centrifuged for progressively increasing periods of time.

It is readily observable that heating the skimmilk to 88° C. for 15 minutes produced no change in the calcium-nitrogen or phosphorus-nitrogen ratios of the complex. This result was unexpected. These samples, however, were centrifuged after a 24-hour holding period. Hilgeman and Jenness *(7)* showed by ultrafiltration methods that the soluble calcium and phosphate were decreased by heating when the filtration was carried out soon after heating, but they returned to the original values when filtration was withheld for 48 hours. Another experiment with the centrifuge showed that heating caused a decrease in the soluble calcium and phosphorus when the milk was centrifuged immediately after heating, i.e., the lines representing the calcium-nitrogen and phosphorus-nitrogen relationships for the heated milk were below those for the raw milk. Therefore, the conclusion of Hilgeman and Jenness that heat precipitation of calcium phosphate is a reversible process is also borne out by the experiments reported here.

A pronounced increase in the calcium-nitrogen and phosphorus-nitrogen ratios in the sediment resulted from adding Na_2HPO_4 to the raw milk. A further

increase in the proportion of these constituents in the sediment resulted from heating a sample after adding Na_2HPO_4. The fact that these lines remain practically straight for the treated samples shows that this additional colloidal calcium phosphate is associated with the caseinate particles.

Calculation of the form of colloidal calcium phosphate in the complex. Estimations of the distribution of the calcium and phosphorus among various phases in the milk, and the form in which the colloidal portion of the calcium phosphate exists, were made from the data obtained in these experiments and from values in the literature. The method was similar to that used by Ramsdell and Hufnagel *(11)*, except that acid-soluble phosphorus was not determined in the present trials. They used this determination for obtaining the organic phosphorus content of casein. The authors assumed the phosphorus content of casein (water and ash-free) to be 0.85%, as given by Eilers *et al. (5)*. At pH 6.7, casein combines with calcium to the extent of 1.07% of the calcium caseinate *(13)*. For convenience the calcium in this form is designated "organic" calcium. Van der Burg *(14)* pointed out that this fraction is molecularly equivalent to the ester-phosphate. In addition to the "organic" form of calcium and phosphorus, both of these elements also exist in a serum or soluble phase and as a colloidal calcium phosphate phase. The latter is the form and quantity to be calculated. The calcium (or phosphorus) in this form is equal to the total analytical amount minus the sum of the serum and "organic" values. The serum values for calcium and phosphorus were obtained by extending the plots in Figures 1 and 2, respectively, to zero casein-nitrogen content. The intercept values were then cor-

TABLE 1

The effects of preheating and of the addition of Na_2HPO_4 on the distribution of calcium and phosphorus in skimmilk

	Raw skimmilk[a]	Raw skimmilk plus 0.15% Na_2HPO_4	Heated skimmilk plus 0.15% Na_2HPO_4
	(%)	*(%)*	*(%)*
Casein nitrogen	0.415	0.415	0.415
Casein	2.648	2.648	2.648
"Organic calcium"	0.0283	0.0283	0.0283
Serum calcium	0.0399	0.0272	0.0146
Total calcium	0.1260	0.126–	0.126
Serum + organic calcium	0.0682	0.0555	0.0429
Colloidal calcium	0.0578	0.0705	0.0831
Organic phosphorus	0.0225	0.0225	0.0225
Serum phosphorus	0.0457	0.0700	0.0573
Total phosphorus	0.099	0.131	0.131
Serum + organic phosphorus	0.0682	0.0925	0.0798
Colloidal phosphorus	0.0308	0.0385	0.0512
Ratio Ca/P	1.88	1.83	1.62
Mole ratio Ca/P	1.45	1.41	1.25
Per cent of total Ca as serum Ca	31.7	21.6	11.6

[a] These figures apply also to the sample which was heated to 88° C. for 15 minutes.

rected for the amount of sediment removed by using the factor 0.972 (the complex amounted to 2.8% of the original skimmilk). The percentage of casein was obtained by multiplying the casein-nitrogen by 6.38. The effects of the heat and addition of Na_2HPO_4 on the distribution of calcium and phosphorus among the three forms are shown in Table 1. The values given are percentages of the original skimmilk.

As explained previously, heating alone gave no change in distribution. The calcium-phosphorus molar ratio in the inorganic colloidal phase of the raw and heated samples was calculated to be 1.45. This value is near the theoretical value for tricalcium phosphate, which is in agreement with Ramsdell and Hufnagel's data *(11)*. A value of 1.41 was calculated for this ratio in the raw sample to which 0.15% Na_2HPO_4 was added. This small difference is probably within the experimental error. However, the ratio of 1.25 obtained for the heated sample containing added phosphate appears to be a significant decrease from the ratio of the control sample. A similar difference was obtained with another sample of milk given the same treatment. This decrease in the ratio of calcium to phosphorus indicates that some dicalcium phosphate was formed when Na_2HPO_4 was added before heating the milk.

The bottom line in Table 1 gives the per cent of the total calcium in the serum phase. The value of 31.7% for the control sample is in good agreement with the values yielded by the dialysate, rennet, and papain methods employed by Pyne *(9)* and also by the ion-exchange procedure used by Baker *et al. (2)*. About one-third of the serum calcium $\left(\frac{31.7 - 21.6}{31.7}\right)$ was removed from this phase by adding 0.15% Na_2HPO_4 to the raw milk. About two-thirds $\left(\frac{31.7 - 11.6}{31.7}\right)$ was removed by heating to 88° C. after adding the phosphate.

SUMMARY

The molar ratio of calcium to phosphorus in the so-called "colloidal calcium phosphate" of the casein complex was calculated to be approximately 1.5 in raw skimmilk. This ratio was reduced to 1.25 when 0.15% Na_2HPO_4 was added before heating to 88° C. for 15 minutes, indicating that some diphosphate was formed. The composition of the sedimented complex with respect to calcium, phosphorus, and nitrogen was practically constant regardless of particle size. The calcium in the serum phase was reduced to about one-third its original value by adding 0.15% Na_2HPO_4 before preheating skimmilk.

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ITALIAN CHEESE RIPENING. IV. VARIOUS FREE AMINO AND FATTY ACIDS IN COMMERCIAL PROVOLONE CHEESE[1,2]

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Department of Dairy Technology, Institute of Nutrition and Food Technology
The Ohio State University, Columbus

AND

Ohio Agricultural Experiment Station, Wooster

A need for more complete information on the complex ripening process of cheese is now well recognized, and research is proceeding in this direction. To this end, determinations of specific end-products of cheese ripening have been given attention in recent years by several investigators *(5, 7, 8, 11)*. However, most of these studies are limited in that they have involved measurements of a single class of chemical compounds, e.g., either protein degradation or fat degradation products, whereas the characteristics of ripened cheese result from complex chemical interrelationships. Therefore, a complete knowledge of ripening changes cannot be obtained until studies of a broad nature are conducted in which individual compounds of various classes are considered simultaneously.

General information concerning the protein and fat degradation in the ripening of Italian cheese has been reported earlier *(3, 4)*. This paper presents the results obtained in a study dealing specifically with the identification and measurement of various fatty acids and amino acids in Provolone cheese.

PROCEDURE

Two phases were involved in this investigation. The first phase was concerned with the analysis of ten commercial Provolone cheeses at 9 months of age for various free amino and fatty acids. The second phase involved analysis for free butyric and glutamic acids of 20 commercial Provolone cheeses of various ages and of different intensities of characteristic flavor.

The cheeses of the same age were manufactured by various commercial companies, each using its own specific method. The manufacture of each lot of cheese was observed and complete manufacturing data were obtained by one of the authors.[3] Although these cheeses were manufactured at different factories, the processes were similar. The principal variables were the type and source of enzyme preparation and the starter culture. In this paper the term enzyme preparation means the rennin and lipase-containing preparation(s) added to the milk.

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[3] W. J. Harper.

At each plant, samples of starters were collected and sent directly to the Dairy Products Section, Eastern Utilization Research Service, USDA, for bacteriological analyses. The cheeses were transported to The Ohio State University, where they were cured at 50 °F. and 75% relative humidity for 9 months. These were considered to be aged cheeses, and analyses were made of cheese of the same age in order to determine what variations existed in the end-product content of the various samples.

All cheeses were judged organoleptically by a panel of experienced judges. Particular attention was given to the intensity of characteristic flavor development of the cheese.

The cheeses were sampled by removing a 2-in. cross section from the cheese, discarding the outer ¼ in. of rind and grinding the sample with a rotary grater. Moisture and water-soluble nitrogen were determined as outlined previously *(3)*. For amino acid analyses a 100-ml. aliquot of the water extract *(3)* was concentrated to 10 ml. under vacuum (20 mm.) at 5 °C. Antifoam B (Dow-Corning) was used to eliminate foaming. This product did not affect the results of amino acid analyses.

Five grams of cheese were analyzed for acetic, propionic, and butyric acids by the direct chromatographic procedure of Harper *(2)*. This method utilizes a column of buffered, acidic silica gel, and butanol-chloroform mixtures to effect the separation and quantitative elution of these compounds.

The buffered paper chromatographic method of McFarren *(9)* was used to separate the amino acids. Alanine, aspartic acid, glutamic acid, glycine, and threonine were separated by using phenol as the mobile phase and pH 12.0 buffer as the nonmobile phase. Benzyl-butyl alcohol was used as the mobile phase with pH 6.2 buffer for the nonmobile phase in the separation of the leucines. Histidine, α-amino butyric acid, methionine, phenylalanine, and valine utilized M-cresol for the mobile phase and pH 8.4 buffer for the nonmobile solvent. The solvents used as the mobile phases were redistilled prior to each chromatographic analysis to remove impurities, which would affect the results.

Amino acid separations were conducted at a constant temperature of 21° ± 1 °C. Filter paper-lined chambers were used to maintain a constant humidity, and they were equilibrated for 24 hours before beginning the separation. A standard concentration of each individual known acid was chromatographed in all experiments to serve as a control.

Quantitative analysis of the amino acids was achieved by modifying slightly the ninhydrin technique of Moore and Stein *(10)* as applied to paper by Honer and Tuckey *(5)*. All lots of water and alcohol were boiled prior to use in order to eliminate ammonia contamination from interfering in color development. After color development was achieved, dilution was made by the addition of 2.0 ml. of boiled distilled water.

All amino acid analyses were made within 12 hours after the preparation of the water extracts, the extracts being held at −20° C. until used. Tests showed that the free amino acid content of the concentrate did not change during 24 hours of storage at this temperature but did change on longer storage.

Trials were made to establish reliability of the amino acid methods. The R_f values for the pure amino acids were found to be reproducible and to agree with the published values except for histidine and valine. Histidine gave an R_f value of 0.40 ± 0.01, and valine had an R_f value of 0.49 ± 0.01. The R_f value of α-amino butyric acid that was not reported previously *(9)* was found to be 0.34 ± 0.01. In all trials, the R_f values obtained for any amino acid separated from cheese extracts did not vary by more than ± 0.02 R_f. This indicates the improbability of peptide interference—a fact demonstrated for one sample by acid hydrolyzing and rechromatographing the free acids in the chromatogram.

Recovery trials revealed that the cheese extracts did not affect the results of the paper chromatographic analysis, since recovery of added amino acids varied from 91 to 98% for all of the acids studied.

RESULTS AND DISCUSSION

The enzyme preparation, type of starter organism, flavor description, age, moisture, and water-soluble nitrogen contents of the ten samples of 9-month-old commercial Provolone cheeses are given in Table 1.

TABLE 1

Composition of commercial 9-month-old Provolone cheese

Sample No.	Source and type of enzyme preparation	Description of flavor[a]	Moisture	Water-soluble nitrogen as % of total nitrogen	Organism found in starter
			(%)	*(%)*	
1	Domestic calf paste	Cheddar flavor CF-0	40.5	33.15	*L. lactis* mycoderm, yeast
2	Imported calf paste	Mild CF-1	38.0	21.5	*L. bulgaricus* lactose fermenting yeast
3	Domestic calf rennet paste	Mild CF-1	35.7	14.6	*L. bulgaricus* yeast
4	Domestic calf rennet paste	Mild CF-1	38.0	20.8	*L. lactis* mycoderm, yeast
5	Unknown	Mild CF-1	33.2	19.3	*L. bulgaricus*
6	Calf gland enzyme product	CF-2	34.5	13.8	*L. lactis*
7	Kid rennet paste	CF-2	40.2	31.9	*L. lactis* *S. durans*
8	Kid gland enzyme	Piquant CF-3	37.8	23.5	*L. lactis* and lactose fermenting yeast
9	Kid rennet paste	Good CF-3	34.5	17.5	*L. bulgaricus*
10	Kid gland and calf gland enzyme	Sharp, Ideal CF-4	33.9	22.4	*S. thermophilus*

[a] CF = characteristic Provolone flavor; intensity range from 0 to 4.

TABLE 2

Free amino acid content of aged commercial Provolone cheese

Sample No.	Description of flavor[b]	Glutamic acid	Leucines	Valine	Alanine	Aspartic acid	Glycine	Methionine	Histidine	Threonine	Amino butyric
		(mg. per gram cheese, dry weight)[a]									
1	CF-0	14.9	16.0	4.7	8.1	4.4	+	1.3	−	−	−
2	CF-1	3.4	11.5	0.1	1.5	1.2	+	+	−	−	−
3	CF-1	2.9	8.5	3.0	1.7	2.3	0.7	+	−	+	−
4	CF-1	5.7	11.6	4.5	3.8	2.4	0.8	0.9	−	−	−
5	CF-1	3.5	6.2	2.1	1.5	+	0.4	+	−	−	−
6	CF-2	8.0	11.6	6.4	2.4	4.4	1.7	+	−	+	−
7	CF-2	8.8	15.0	2.2	2.1	3.1	+	+	−	−	−
8	CF-3	8.4	10.9	5.3	3.1	4.2	1.4	+	+	+	+
9	CF-3	5.4	10.7	3.9	1.5	5.3	0.8	.89	−	+	−
10	CF-4	8.3	12.1	6.8	3.5	4.6	1.3	.58	−	+	−

[a] + indicates presence of the acid but in quantities > 0.1 mg. per gram solids.

[b] CF = characteristic Provolone flavor; intensity range from 0 to 4.

Results reveal that the flavor of the cheeses varied from Cheddar-like to a sharp piquant characteristic Provolone flavor. These data verify the previous report that the flavor was not related to per cent of water-soluble nitrogen but was definitely related to the type of enzyme preparation used.

The results of the free amino acid determinations are given in Table 2. The separation of leucine and isoleucine was incomplete in some samples; therefore, these acids are reported together as combined "leucines."

Qualitatively, the free amino acid content of the various samples was similar. Histidine, α-amino butyric acid, and threonine were generally not present or were in such low concentrations that quantitative measurement was not possible (0.1 mg. per gram cheese solids). The other amino acids were found in all ten samples. However, the concentrations of these acids varied greatly among the different samples of cheese of the same age. For example, the glutamic acid content varied from 2.9 to 14.9 mg. per gram of cheese solids, the valine content varied from 0.1 to 6.8 mg., and the alanine concentrations were between 1.5 and 8.1 mg. The concentration of combined leucine and isoleucine showed the least variation, varying from 6.2 to 16.0 mg. per gram solids.

The amino acids were not present as free acids in the cheese in the same ratio as they are found in the original intact casein. Free glutamic and aspartic acids were generally present in a relatively lower concentration and the leucine and glycine in relatively higher concentrations than they appear in casein. The work of Bassett *et al. (1)* indicates that these differences in ratios might be best explained by random protein degradation of the casein accompanied by specific chemical or enzymatic degradation of the amino acids. The formation of tyramine in Cheddar cheese *(6)* and decreases in free glutamic acid and alanine during certain phases of Cheddar cheese ripening *(5)* serve as examples of such changes. Such secondary reactions would be expected to influence accumulation of end-products and, thus, flavor development.

There was no consistent correlation between the total free amino acid content and the intensity of characteristic flavor. The cheese having the least characteristic flavor had the highest total free amino acid content (Sample 1). However, there was generally a direct relationship between the concentrations of free glutamic acid, alanine, valine, aspartic acid and flavor intensity. For example, the four cheeses with a CF-1 flavor rating contained an average of 3.3 mg. of glutamic acid, 2.4 mg. of valine, and 1.5 mg. of aspartic acid, whereas the five cheeses with a CF-2 or more had an average of 7.6 mg. of glutamic acid, 4.8 mg. of valine, and 4.3 mg. of aspartic acid per gram solids.

The variations in free amino acid content could not be related to the type of rennet paste used in the manufacture of the Provolone cheese. In contrast, a general relationship was observed between the free amino acid content of the cheese and the major lactic acid starter organisms present in the starter culture. Although the samples are too few to give conclusive evidence, the data in Table 3 reveal the average free amino acid content of cheese made with *Lactobacillus lactis* to be much higher than when *L. bulgaricus* was the major starter organism. Tittsler *(12)* reported that *L. bulgaricus* was not found in the cheese after brine

TABLE 3

Relationship of starter organisms to the concentration of free amino acids in commercial Provolone nine months of age

Amino acid	*Lactobacillus lactis*			*Lactobacillus bulgaricus*		
	No. of samples	Concentration of free free amino acids in mg/g solids		No. of samples	Concentration of free free amino acids in mg/g solids	
		Range	Average		Range	Average
Glutamic acid	5	5.7-14.9	9.2	4	2.9-5.4	3.8
Aspartic acid	5	2.4-4.4	3.7	4	0.1-5.3	2.2
Leucines	5	10.9-16	11.0	4	6.2-10.7	9.22
Valine	5	2.2-6.4	4.6	4	0.1-3.9	2.22
Alanine	5	2.4-8.1	3.9	4	1.5-2.4	1.6

saltings, whereas *L. lactis* was found to persist during the ripening period. This possible relationship between the type of starter organism and the accumulation of free amino acids is receiving further study.

Free fatty acids. The free acetic, propionic, and butyric acid content of the ten samples of Provolone are given in Table 4. The butyric acid content varied from 0.18 to 4.2 mg. per gram of cheese solids, whereas the free acetic acid ranged from 0.16 to 0.47. The type of starter could not be related to the free fatty acid content of the different cheese samples. Of the three acids considered, only the free butyric acid was definitely related to the type enzyme product used in making the cheese. The domestic rennet pastes, which generally have been purified, are associated with the less-flavored cheeses and with low free butyric acid values.

The acetic and propionic acids were related to the characteristic flavor of the Provolone cheeses in that two samples criticized as being "acidy" contained the highest concentration of acetic acid. The concentration of free butyric acid

TABLE 4

The lower fatty acid content of aged commercial Provolone cheese

Sample No.	Enzyme product	Flavor	Acetic	Propionic	Butyric
			(mg. per gram cheese solids)		
1	Purified calf rennet paste	Cheddar-like, CF-0[a]	0.16	0.09	0.18
2	Purified calf rennet paste	Mild, acidy, CF-1	0.19	0.22	1.05
3	Imported calf rennet paste	Mild, CF-1	0.18	0.11	1.731
4	Purified calf rennet paste	Mild, CF-1	0.19	0.66	0.50
5	Unknown	Mild, CF-1	0.16	0.15	0.581
6	Glandular calf enzyme product	Good, CF-2	0.16	0.13	1.28
7	Kid rennet paste	Good, CF-2	0.19	0.16	2.496
8	Kid glandular enzyme	Piquant, CF-3	0.13	0.21	3.1
9	Kid rennet paste	Sharp, CF-3	0.22	0.20	2.83
10	Glandular calf and glandular kid enzyme products	Sharp, sl. acidy	0.47	0.25	4.34

[a] CF is intensity of characteristic Provolone cheese flavor; none is CF-0, maximum is CF-4.

TABLE 5

Relationship of characteristic flavor to the free butyric and free glutamic acid content of Provolone cheese

CF[a]-0		CF-0-1		CF-1		CF-2		CF-3		CF-4	
Butyric acid	Glutamic acid	Butyric acid	Glutamic acid	Butyric acid	Glutamic acid	Butyric acid	Glutamic acid	Butyric acid	Glutamic acid	Butyric acid	Glutamic acid
(mg. per gram cheese solids)											
0.19	14.00	1.6	1.4	2.2	2.1	1.5	4.4	2.6	5.8	4.4	8.3
0.51	0.68	1.8	1.1	1.0	3.4	2.6	3.7	2.3	4.0	5.1	10.8
0.55	1.30	1.3	1.9	1.6	2.8	2.7	8.8	3.1	8.4		
0.55	4.1			1.7	2.0	1.2	8.0	2.8	5.4		
0.58	1.60			0.8	5.7	2.1	4.3				
0.88	0.78			0.58	3.5	2.5	8.8				
1.10	0.51										
1.30	0.51										
1.60	0.50										

[a] CF = characteristic flavor. Recorded from 0 to 4.

was found to be directly related to the intensities of the desired flavor of the cheese, with cheese having higher butyric acid values generally having the high characteristic flavor scores. Samples 2 and 3 were exceptions to this relationship.

The interrelationship between butyric and glutamic acid and flavor of Provolone cheese. A possible interrelationship between the concentrations of free butyric and free glutamic acids and the intensity of characteristic flavor was suspected on the basis of the values presented in Tables 2 and 4. In general, the concentrations of both acids were much greater in cheeses that had high flavor scores than in those that had a low flavor score.

In order to study further a possible interrelationship between butyric and glutamic acids, 20 additional samples of Provolone cheese were analyzed for butyric acid and glutamic acid. These cheeses were of various ages from 30 days to 18 months. Each cheese was judged organoleptically. In Table 5 the butyric and glutamic acid values for these 20 samples and the 10 samples previously studied are arranged according to the flavor intensity of the cheese.

The data indicate a definite relationship between the concentration of the free glutamic and butyric acid and the intensity of characteristic flavor of the cheese. The concentration of each of the acids apparently had to reach a threshold value in concentration before the desired flavor was evident. In cheese with CF-1, this threshold value was approximately 2.0 mg. of glutamic acid and 1.0 mg. of butyric acid. Some samples with a CF-0 contained concentration of butyric acid greater than 1.0 mg. per gram, but the glutamic concentration was below the threshold value of 2.0 mg. Two cheeses with more than 2.0 mg. of glutamic acid contained less than 1.0 mg. of butyric acid and possessed no desired flavor. The cheeses with a CF-0 and CF-1 contained a concentration of butyric acid greater than the threshold value, but the glutamic acid concentration was slightly below 2.0 mg.

Cheeses with a CF-2 contained at least 3.5 mg. of glutamic acid and 1.5 mg. of butyric acid. This is approximately a 2:1 ratio. At least 4.0 mg. of glutamic acid and 2.3 mg. of butyric acid were present in cheese with a CF-3. The two cheeses with a CF-4 contained the highest concentration of butyric acid and relatively high concentrations of free glutamic acid. Here again the ratio of free glutamic to free butyric acid was approximately 2:1.

SUMMARY

The free amino acid and free fatty acid content of commercial Provolone cheeses of the same age exhibited wide variations. The free fatty acid content varied in relation to the type of enzyme product used in its manufacture, whereas the free amino acid content was apparently related to the type of starter organism.

Purified glandular lipases were found to produce cheese of at least comparable quality to that made with crude rennet pastes. The importance of the lipase in these enzyme products has been shown to be related directly to the production of butyric acid.

An interrelationship was noted between the concentration of free butyric acid and free glutamic acid (per gram of cheese solids) and desired flavor intensity. About 1 mg. of free butyric acid and 2 mg. of free glutamic acid were found in cheeses in which characteristic flavor was noted. The concentration of each increased with flavor intensity, maintaining a ratio of approximately 1:2 of butyric to glutamic acid.

The results of this study emphasize the value of measuring individual degradation products of both fats and proteins simultaneously. More complete analyses for various types of compounds appear to be a valuable approach for future studies.

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ITALIAN CHEESE RIPENING. V. VARIOUS FREE AMINO AND FATTY ACIDS IN COMMERCIAL ROMANO CHEESE[1,2]

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The gross chemical changes that occur during the ripening of Romano cheese have been reported in previous papers of this series *(2, 3)*. Although these investigations indicate that both fat and protein degradation is important in the ripening of this cheese, no information was presented as to the specific chemical compounds that are related to characteristic flavor development. In another paper *(4)* data were presented for various free amino acids in Provolone cheese; they showed that both free butyric and free glutamic acid are associated with the development of the characteristic flavor of this cheese. A related study was conducted on Romano cheese and the findings are presented in this paper.

PROCEDURE

Fourteen samples of commercial Romano cheese of different ages were obtained from various domestic factories making Italian cheese. Seven of the cheeses were 4 months old, three were 10 months old, three were 18 months old, and one was 26 months of age.

The manufacturing of the seven 4-month-old cheeses was observed by visiting each of the five factories which made these cheeses. The methods used by the different plants varied slightly but all were considered as being representative commercial procedures. After salting, the cheeses were cured at The Ohio State University under the same conditions of temperature (50° F.) and humidity (70% relative humidity).

The other seven cheeses were obtained directly from the manufacturers after they had been cured under the commercial conditions in the factories where they had been made. A history of each cheese was obtained.

The cheeses were sampled by removing a wedge about 2 in. wide at the outer edge. The outer ¼ in. of rind was discarded, and the cheese was ground in a rotary hand grater, packed in air-tight containers, and stored at −15° C. until analyzed. Analyses for moisture and water-soluble nitrogen were made within 48 hours in the same manner as previously reported *(4)*.

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[2] Journal Article 39-55, Ohio Agricultural Experiment Station, Wooster.

TABLE 1

General characteristics of Romano cheese

Sample No.	Age	Flavor		Source and type of enzyme product[b]	Moisture	Water soluble nitrogen
		Intensity	Comment			
	(mo.)	*(C.F.)*[a]			*(%)*	*(% of total N)*
1	4	0.5	Mild	Purified calf and kid rennet paste	33.1	15.4
2	4	1	Acidy	Crude mixed calf and kid rennet paste	35.2	13.4
3	4	2	Clean	Kid glandular preparation	34.4	23.4
4	4	2	Sl. unclean	Kid rennet paste	34.7	17.2
5	4	2	Sl. bitter	Calf rennet paste	33.8	14.8
6	4	3	Sharp, goaty	Kid glandular preparation	31.8	12.9
7	4	3	Piquant, sharp	Lamb glandular preparation	35.6	20.9
8	10	1	Mild, acidy	Purified calf rennet paste	32.8	20.4
9	10	1	Medicinal, mild	Calf and kid glandular preparation	33.8	24.4
10	10	2½	Clean	Kid glandular preparation	34.0	21.0
11	18	1	Mild	Rennet extract	33.6	21.3
12	18	2	Sl. sweet	Calf glandular preparation	34.0	18.0
13	18	3	Sharp, goaty	Kid glandular preparation	33.6	—
14	26	4	Ideal	Kid rennet paste	32.9	15.4

[a] C.F. = characteristic flavor; 0 = no flavor; 4 = ideal, highest characteristic flavor.

[b] Rennet extract was used with all glandular preparations.

A portion of the water extract, which had been prepared according to the method of Sommer and Harper *(7)*, was concentrated ten to one under vacuum (20 mm.) at low temperature (5° C.). This concentrate was used within 24 hours for amino acid analyses. Analyses for representative free amino acids (aspartic acid, glutamic acid, glycine, alanine, threonine, histidine, valine, methionine, and the combined leucines) were made by the paper chromatographic procedure of McFarren *(6)* as described earlier *(4, 5)*.

Analyses were made also for acetic, propionic, and butyric acids with 5 g. of cheese by a direct silica gel chromatographic procedure *(1)*.

RESULTS

The ages, characteristic flavor intensities, sources and types of enzyme utilized in manufacture, moisture, and water-soluble nitrogen content are given in Table 1. The samples are arranged in order of increasing age, and those of the same age are arranged in order of increasing flavor intensity. As reported in a previous paper *(3)*, the flavor intensity was generally related to the type of enzyme products used in the manufacture of the cheese. Also, the results confirmed those previously reported for Provolone cheese in that there was no definite relationship between the water-soluble nitrogen and the characteristic flavor intensity of the Romano cheese *(3)*. The highest water-soluble content was found in Sample 9, which had one of the lowest flavor intensities, whereas the lowest water-soluble nitrogen value was found in Sample 6, which had a relatively high degree of characteristic flavor. The moisture contents of the cheeses were in general agreement with those previously reported.

The results of the analyses for the various free amino acids are presented in Table 2. The samples are arranged in the same order as in Table 1. Qualitatively, all samples contained aspartic acid, alanine, glycine, glutamic acid, leucines, and valine, whereas some samples did not have any detectable quantities of histidine, methionine, and threonine.

The quantitative values reveal wide variations in the concentration of a given acid in cheeses of the same or different ages. The seven 4-month-old cheeses generally had a lower concentration of aspartic acid, glycine, and methionine, whereas the seven samples 10 months old or older contained less histidine and threonine. On the other hand, the concentrations of alanine, glutamic acid, leucines, and valine did not appear to be related to variations in age of the cheese. With the exception of glutamic acid, none of the amino acids revealed any definite relationship to characteristic flavor development. Glutamic acid was generally high in samples receiving a relatively high flavor intensity score. For example, the cheeses with a CF-2 or higher had a glutamic acid range of 4.8 to 6.3 mg. per gram solids with an average value of 5.5; whereas those with a CF less than 2 had a glutamic acid range from 2.1 to 6.0 mg. per gram solids and an average of 3.4 mg. per gram solids.

The amounts of various free fatty acids in the 14 commercial Romano cheeses are given in Table 3. Acetic, propionic, butyric, and the group designated as "higher" acids were present in all samples, although the concentration varied

TABLE 2

The free amino acid content of commercial Romano cheese

Sample No.	Age	Flavor intensity	Aspartic acid	Alanine	Glycine	Glutamic acid	Histidine	Leucines	Methionine	Threonine	Valine
	(mo.)	*(C.F.)*[a]	*(mg. per gram cheese solids)*								
1	4	0.5	+	1.2	+	3.8	7.0	8.2	+	1.5	3.0
2	4	1	0.6	1.1	+	2.1	7.6	8.3	0	0	2.6
3	4	2	0.9	1.6	+	4.8	6.0	9.7	+	1.2	3.9
4	4	2	+	2.1	0.7	5.3	4.2	9.1	0.3	0.8	3.1
5	4	2	1.1	0.8	+	5.0	2.8	9.6	+	0	3.5
6	4	3	+	1.6	+	—	4.1	7.5	0.2	1.8	3.7
7	4	3	1.7	1.3	0.8	5.7	3.6	8.9	+	0.9	3.6
8	10	1	2.0	—	1.3	3.0	8.0	9.7	0	1.8	4.2
9	10	1	1.4	1.7	0.5	2.4	6.0	9.6	0	2.0	5.5
10	10	2.5	1.3	1.1	1.2	6.3	0	12.5	+	0	3.4
11	18	1	2.9	1.2	+	6.0	0	9.9	0.73	0	3.9
12	18	2	1.6	3.5	0.9	5.7	4.9	9.0	0.7	0	4.0
13	18	3	2.6	4.0	0.8	5.4	2.9	10.7	0.7	0	4.3
14	26	4	0.9	1.2	+	5.6	0	8.3	0.6	0	3.6

[a] C.F. = characteristic flavor; 0 = no flavor; 4 = ideal, highest characteristic flavor.

TABLE 3
The free fatty acid content of commercial Romano cheese

Sample No.	Age	C.F.[a]	Acetic	Propionic	Butyric	Higher[b]
	(mo.)		*(mg. per gram cheese solids)*			
1	4	0.5	0.2	0.05	0.8	2.7
2	4	1	0.86	0.21	1.7	6.4
3	4	2	0.20	0.16	1.5	5.0
4	4	2	0.27	0.18	1.8	6.1
5	4	2	0.31	0.23	1.1	4.7
6	4	3	0.26	0.14	3.0	8.1
7	4	3	0.29	0.22	3.2	6.3
8	10	1	0.56	0.30	0.9	2.3
9	10	1	0.32	0.26	2.1	8.4
10	10	2.5	0.24	0.18	3.6	10.1
11	18	1	0.15	0.11	0.8	3.1
12	18	2	0.41	0.34	2.8	9.3
13	18	3	0.30	0.24	6.4	10.9
14	26	4	0.41	0.26	7.1	14.7

[a] See footnote on Table 1.

[b] Includes all fatty acids above 5 carbon atoms; a molecular weight of 150 was assumed to calculate higher acid concentration.

widely among the different samples. The amounts of acetic and propionic acids could not be related to the age of the samples and were present in lower concentrations than butyric acid. Generally, the butyric acid content of the 4-month samples was less than that of the samples 10 or more months of age. Similarly, the higher fatty acids were usually higher in the older cheeses. The exceptions to the age relationships, Samples 8 and 11, can be explained on the basis of the enzyme system used in manufacture. Sample 11 was made with rennet extract only, and Sample 8 was made with a purified rennet paste. These samples contained 0.8 and 0.9 mg. butyric acid per gram cheese solids, respectively. Sample 1, which was the only other sample made with a purified rennet paste, was also the only sample with less than 1 mg. of butyric acid per gram of cheese solids. This relationship of enzyme product to the butyric acid and higher acid values was in accordance with findings in earlier studies involving the volatile acids formed in Romano cheese *(3)*.

Generally, the butyric acid content of the Romano cheese was related to the characteristic flavor intensity. However, there were more exceptions to this relationship than previously shown between butyric acid and Provolone cheese flavor *(4)*. Cheeses with a CF-1 contained from 0.8 to 2.1 mg. of butyric acid per gram cheese solids, whereas cheese with a CF-2 contained from 1.1 to 2.8 mg. of butyric acid. The cheeses with a CF value above 2 contained from 3.0 to 7.1 mg. of butyric acid. The higher fatty acids also were found to be related generally to the flavor intensity with such exceptions as Sample 2 with a CF-1 and 6.4 mg. of higher acids, as compared to Sample 7 with a CF-3 and 6.3 mg. of higher acids. Sample 9 with a CF-1 contained 8.4 mg. of higher acids as compared to Sample 6 with a CF-3 and 8.1 mg. of higher acids.

In the previous paper of this series dealing with Provolone cheese, a definite relationship was found between glutamic acid and butyric acid content as related

TABLE 4

The relationship of characteristic flavor to the free butyric and glutamic acid content of commercial Romano cheese

C.F.[a] 0.5 and 1.0		C.F. 2.0 and 2.5		C.F. 3.0 and 4.0	
Butyric	Glutamic	Butyric	Glutamic	Butyric	Glutamic
(mg.)	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*
0.8	3.8	1.1	5.0	3.0	5.7
0.9	3.0	1.5	4.8	3.2	5.4
0.8	6.0	1.8	5.3	6.4	5.6
1.7	2.1	2.8	5.7	7.1	5.6
2.1	6.3	3.6	2.4		

[a] C.F. = characteristic flavor. Recorded from 0 to 4.

to flavor intensity *(4)*. In order to determine if such a relationship might exist also in Romano cheese, the butyric acid and glutamic acid values are compared to flavor intensity in Table 4. Some of the exceptions to the relationship of either glutamic or butyric acid to flavor intensity can be explained by the data in this table. For example, in the first column the cheese with a glutamic acid value of 6.0 had a corresponding butyric acid value of 0.8. In the same column, the fourth sample had a relatively high butyric acid value (1.7) but a relatively low glutamic acid value. However, there is no clearly defined relationship between glutamic acid and butyric acid and flavor development, as was noted for Provolone cheese.

DISCUSSION

The results of this investigation on Romano cheese are in general agreement with those for Provolone cheese, which were reported previously *(4)*. These two cheeses are closely related in that they depend upon the same bacterial starters and on the same lipolytic enzymes and differ primarily in the heat treatments which they receive during manufacture. The free amino acids and free fatty acid contents of Romano cheese were generally higher than those of Provolone cheese, which is probably explained by the high heat treatment which Provolone cheese received during molding.

In the present study the variability in the concentrations of the free fatty acids could be related to the type of lipase system used in the manufacture of the cheese, confirming the earlier results on Provolone *(4)*. In the present study, insufficient data were available concerning the starter cultures used in the cheese to permit determining whether or not any relationship existed between the type of starter organisms and the accumulation of the various free amino acids. However, the microbial flora is undoubtedly important in determining which amino acids accumulate during the cheese ripening, and further studies are being directed to the bacterial enzymatic mechanisms involved in the ripening of Romano cheese.

Much time and effort have been expended by many research workers to determine the chemical compounds related to flavor development of cheese. Ideally, analyses should be made simultaneously for as many different types of com-

pounds as possible, but the time limitations have made this impossible. Therefore, analyses generally have been limited to one group of compounds. The approach used in this study has been to study representative compounds of different groups. The interrelationship observed between an amino acid (glutamic) and a fatty acid (butyric) suggests that analyses including compounds of other groups, such as carbonyls, alcohols, and amines, could provide further information concerning the cheese ripening process.

The fact that a relationship between flavor and the butyric acid–glutamic acid content exists does not prove that such compounds are necessarily flavor compounds. They may be precursors of flavor constituents or merely associated with other flavor compounds. In Provolone cheese, there was a definite relationship between the flavor intensity of 30 samples of cheese and the combined butyric–glutamic acid content. In the present study, there is an indication of this relationship, but the irregularity of the results does not permit a definite conclusion in this respect. The most probable explanation is that a third compound or compounds may be interrelated with the butyric and glutamic acids. This hypothesis is strengthened by the fact that the group of higher acids appears to be related to the flavor of this cheese.

Both butyric and glutamic acid could be subject to further degradation to form other compounds, which may explain the role of the former compounds in cheese flavor. On the other hand, it is possible that amino acids and fatty acids might form a salt complex which would have flavor imparting characteristics. The butyric-glutamic relationship in Provolone and the propionic-proline relationship in Swiss cheese suggest this possibility *(4, 5)*.

SUMMARY

Various free amino and fatty acids were measured in 14 samples of commercial Romano cheese. The amounts of some of the free amino acids could be related to the age of the cheese, but only the free glutamic acid could be related to the flavor intensity of the cheese. Acetic and propionic acids were not associated with either the age of the cheese or flavor intensity, whereas both the higher acids and butyric acid were generally related to age and the flavor intensity of cheese of the same age.

In general, the free butyric acid and higher fatty acid contents were dependent upon the type of enzyme product used in the manufacture of the cheese, whereas the free amino acid concentrations were not associated with the lipase product added to the milk.

The results of this study on Romano cheese are in general agreement with those presented earlier for Provolone. The major difference noted was that the amounts of free acids for cheese of the same relative flavor intensity are much higher in the Romano cheese. There were wide variations in the amounts of a given free amino or fatty acid in cheese of the same age. This may be attributed to the differences in manufacturing practices in the various factories making Romano cheese.

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SWITCHBACK TRIALS FOR MORE THAN TWO TREATMENTS[1]

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It is often desirable to take advantage of the sensitivity of switch-back or double-reversal trials when doing research with dairy cattle. Basically, however, these designs permit the comparison of only two treatments, and this has imposed some limitation on their usefulness. The present paper is devoted to switch-back designs that permit comparisons of three or more treatments.

The basic switch-back design is as follows :

Experimental period	Treatment sequence	
	1	2
1	1[a]	2
2	2	1
3	1	2

[a] The numbers in the heart of the table refer to the treatments being compared.

The treatment pattern results in particularly sensitive comparisons, because it permits the elimination from error of *(a)* the period effects due to changes in environment, *(b)* the between-cow variation in production level, and *(c)* most of the between-cow variation in slope of the lactation curve.

Brandt *(1)* has elucidated an efficient statistical analysis for switch-back trials. This analysis is also described by Snedecor *(3)*. Brandt first computes for each cow a quantity

$$D = Y_1 - 2Y_2 + Y_3$$

in which Y_1, Y_2, and Y_3 represent the performances during periods 1, 2, and 3, respectively. To outline Brandt's analysis when the number of cows on the two sequences is equal, let

D_{ij} = performance of the j^{th} cow on the i^{th} sequence $(i = 1, 2;\ j = 1, 2, \ldots r)$

G_i = sum of the D's for the i^{th} sequence

The estimate of the error variance is given by

$$s^2 = \frac{1}{6(2)\ (r-1)} \left\{ \Sigma_i \Sigma_j D^2_{ij} - \frac{G^2_1 + G^2_2}{r} \right\}.$$

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in which $2(r-1)$ = degrees of freedom for error. The divisor 6 is introduced to put the mean square on a per cow per period basis. The advantage for treatment 1 over treatment 2 and its standard error are estimated by

$$\frac{G_1 - G_2}{4r} \quad \text{and} \quad \sqrt{\frac{3s^2}{4r}}$$

respectively. The divisor 4 is introduced to place the contrasts on a per cow per period basis. An extension of Brandt's analysis is used in the present paper.

Seath *(2)* devised a four-sequence switch-back design to study 2×2 factorial patterns of treatments. The design provides a sensitive test of the two main effects but yields a poor test of the interaction. The designs in the present paper provide equal sensitivity for all treatment contrasts.

Taylor and Armstrong *(4)* have pointed out that the switch-back designs can be extended to any number of treatments by setting out the basic switch-back pattern for each possible pair of treatments and have briefly indicated the method of analysis.

The present paper is devoted to designs of the extended type. The analysis is given in detail, and designs of reduced size are discussed along with the introduction of a blocking feature. Missing-value formulas also are given.

THE DESIGNS

The designs combine the switch-back and the balanced incomplete blocks principles. In general, the comparison of p treatments requires $p(p-1)$ treatment sequences (complete designs). If p is odd and 5 or greater, however, designs that require only $p(p-1)/2$ sequences can be used (reduced designs).

Treatment patterns for a selection of complete and reduced designs are given in the following section. Complete designs are shown for 3, 4, and 6 treatments, but only the reduced designs are given for 5, 7, and 9 treatments. The patterns for larger numbers of treatments are easily derived but probably are of limited usefulness because they require so many cows.

For each reduced design a complementary design is easily obtained by writing the second row of the reduced design as the first and third rows of the complement, and the first row of the reduced design as the second row of the complement. The reduced and the complementary designs together form the complete design.

The complete designs are subdivided into $p-1$ blocks of p sequences each, and the reduced designs into $(p-1)/2$ blocks of p sequences each. The rows represent experimental periods, and the columns treatment sequences.

TREATMENT PATTERNS

3 Treatments (complete design):

Block 1			Block 2		
1	2	3	1	2	3
2	3	1	3	1	2
1	2	3	1	2	3

4 Treatments (complete design):

Block 1	Block 2	Block 3
1 2 3 4	1 2 3 4	1 2 3 4
2 3 4 1	3 4 1 2	4 1 2 3
1 2 3 4	1 2 3 4	1 2 3 4

5 Treatments (reduced design):

Block 1	Block 2
1 2 3 4 5	1 2 3 4 5
2 3 4 5 1	3 4 5 1 2
1 2 3 4 5	1 2 3 4 5

6 Treatments (complete design):

Block 1	Block 2	Block 3
1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6
2 3 4 5 6 1	3 4 5 6 1 2	4 5 6 1 2 3
1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6

Block 4	Block 5
1 2 3 4 5 6	1 2 3 4 5 6
5 6 1 2 3 4	6 1 2 3 4 5
1 2 3 4 5 6	1 2 3 4 5 6

7 Treatments (reduced design):

Block 1	Block 2	Block 3
1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7
2 3 4 5 6 7 1	3 4 5 6 7 1 2	4 5 6 7 1 2 3
1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7

9 Treatments (reduced design):

Block 1	Block 2
1 2 3 4 5 6 7 8 9	1 2 3 4 5 6 7 8 9
2 3 4 5 6 7 8 9 1	3 4 5 6 7 8 9 1 2
1 2 3 4 5 6 7 8 9	1 2 3 4 5 6 7 8 9

Block 3	Block 4
1 2 3 4 5 6 7 8 9	1 2 3 4 5 6 7 8 9
4 5 6 7 8 9 1 2 3	5 6 7 8 9 1 2 3 4
1 2 3 4 5 6 7 8 9	1 2 3 4 5 6 7 8 9

USE OF DESIGNS

The first step in using these designs is to allot randomly the treatments to the numbers used in the pattern. Next, the cows are allotted to sequences. If there are enough cows available to start all sequences at the same time, the allotment to the sequences should be completely random. No attempts should be made to group the cows for similarity prior to allotment nor to balance cow differences across treatments. As was noted, the designs and the method of analysis automatically remove from experimental error the between-cow variation in production level and most of the variation in persistency. Thus, balancing on these

factors or on factors associated with them results in no further reduction of experimental error, and a question can be raised as to whether restrictions on allotment reduce the degrees of freedom for error.

For minimum experimental errors all cows should have passed the lactation peak before being placed on experiment, and no cows should have passed midgestation at the close of the experiment. Often, the number of available cows satisfying these conditions is not sufficient to permit starting all sequences of a design at one time. In such an event, a blocking procedure can be used.

One blocking procedure, usable with either the complete or the reduced designs, is to start the blocks shown in the pattern one at the time. The order of starting the blocks and the allotment of the cows to treatment sequences within each block should be at random.

An alternative blocking procedure, usable only with the complete designs, is as follows: Disregard the blocks shown in the patterns and pair off the sequences by treatments. For example, the sequence pairs of the 4-treatment design are:

Pair 1	*Pair 2*	*Pair 3*	*Pair 4*	*Pair 5*	*Pair 6*
1 2	2 3	3 4	4 1	1 3	2 4
2 1	3 2	4 3	1 4	3 1	4 2
1 2	2 3	3 4	4 1	1 3	2 4

If necessary, each pair may serve as a block, but such is not advisable for experiments with few animals because the degrees of freedom for error will be small. Blocks also may be formed by combining two or more pairs. The number of pairs may vary from block to block, but pairmates should not be split, and the allocation of pairs to blocks should be random. Ordinarily an experimenter will know the number of cows that will be available at a certain time and can form the blocks before any cows start on test. For example, two cows might be available on a certain date, six more cows 3 weeks later, and four more 2 weeks after that. It thus would be known that the first block would consist of one sequence pair, the second of three pairs, and the last block of two pairs. The sequence pairs would be randomly allotted to blocks accordingly, and the cows to sequences within blocks.

Even with one cow per sequence, and a maximum subdivision into blocks, all of the design patterns given in this paper provide degrees of freedom for error. In the smaller designs, however, the precision of comparison will be relatively low if only one cow is used per sequence. This is because there will be few replications and few degrees of freedom for error. It seems advisable ordinarily to use enough cows on each sequence to provide a total of at least 8 or 10 degrees of freedom for error. The method of analysis to be outlined permits one or more cows per sequence but requires that the number of cows be the same on all sequences.

THE STATISTICAL ANALYSIS

Preliminary computations: The first step of analysis is to compute for each cow, as did Brandt, the quantity

$$D = Y_1 - 2Y_2 + Y_3$$

in which Y_1, Y_2, and Y_3 represent the performances in periods 1, 2, and 3, respectively. The next step is to compute

M = sum of the D's for all cows on all sequences,

and, if the design is blocked, to compute for each block

B = sum of the D's in the block.

The third step is to compute for each treatment

Q = sum of the D's for the cows receiving the treatment in the first and third periods minus the sum of the D's for the cows receiving the treatment in the second period.

TABLE 1

Computations for the analysis of variance of the D values

Variance source	Degrees of freedom	Sum of squares	
		Abbreviation	Computational formulas[a]
Correction factor	1	CF	$\frac{M^2}{3np(p-1)}$
Total (corrected)	$\frac{np(p-1)}{2}-1$	TSS	$\frac{1}{6}\Sigma_i\Sigma_j D_{ij}^2 - CF$
Blocks (if present)	$v-1$	SSB	$\frac{1}{6}\Sigma_u \frac{B_u^2}{m_u} - CF$
Treatments	$p-1$	SST	$\frac{1}{6np}\Sigma_k Q_k^2$
Error Blocks present	$\frac{np(p-1)-2(p+v)+2}{2}$	SSE	TSS − SSB − SST
No blocks	$\frac{np^2-(n+2)p}{2}$	SSE	TSS − SST

[a] The divisor 6 is used to place the sums of squares on a per cow per period basis.

Analysis of variance: The degrees of freedom and the computational formulas for the sums of squares required in the analysis of variance are shown in Table 1. The parameters of the design and the quantities used in the computational formulas are defined as follows:

p = the number of treatments

n = $\left\{\begin{matrix} r \text{ in a reduced design} \\ 2r \text{ in a complete design} \end{matrix}\right\}$ where r = the number of cows per treatment sequence

v = the number of blocks in the design

m_u = the number of cows in the u^{th} block

D_{ij} = the D value for the j^{th} cow on the i^{th} treatment sequence

B_u = the B value for the u^{th} block

Q_k = the Q value for the k^{th} treatment.

Mean squares may be computed and the F test for treatments made in the usual fashion.

Treatment means: The mean for the k^{th} treatment is obtained as

$$\overline{\overline{Y}} + Q_k/2np$$

in which $\overline{\overline{Y}}$ is the grand mean performance in the experiment, i.e., the mean of the original data, not the mean of the D's.

The standard error of a treatment difference is $\sqrt{3s^2/np}$, where s^2 = error mean square.

Analysis when there are missing or abnormal performances: Occasionally, a cow started on experiment may not finish, or her production during one or more experimental periods may be abnormal owing to accountable factors. When this occurs, it will be necessary to provide a D value for that cow in order to apply the computational formulas previously given for the analysis. If the design is blocked, the missing D value is obtained by the formula,

$$\frac{npB'_s + m_s(Q'_{sf} - Q'_{sc})}{np(m_s - 1) - 2m_s}$$

in which n and p are defined as previously and

m_s = the number of cows intended to be in the block in which the missing value occurs

B'_s = the sum of the D's for the non-missing cows in the block

Q'_{sf} = the Q value for the treatment occurring in the first and third periods on the missing cow, computed by letting the missing D be zero

Q'_{sc} = the Q value for the treatment occurring in the second period on the missing cow, computed by letting the missing D be zero.

If the design is not blocked the formula becomes

$$\frac{2M' + (p-1)\ (Q'_{sf} - Q'_{sc})}{np^2 - (n+2)p}$$

in which M' = the grand sum of the non-missing D's, and the other quantities are defined as before.

A COMPUTATIONAL EXAMPLE

Because no data from feeding trials employing the present designs are yet available, uniformity data will be used to illustrate the calculations. The data simulate an experiment comparing three treatments with 12 cows. To illustrate the blocking feature it is assumed that six of the cows were started at one time with the complete design, and that the remaining six cows were started at later times in two groups of three, each group being assigned to one block of the

TABLE 2
The data and preliminary computations

Cow	Treatment and pounds FCM production per day			D Value
	Period 1	Period 2	Period 3	
Block 1:				
1	(1) 34.6	(2) 32.3	(1) 28.5	−1.5[a]
2	(2) 22.8	(3) 21.0	(2) 18.6	−0.6
3	(3) 32.9	(1) 33.1	(3) 27.5	−5.8
4	(1) 48.9	(3) 46.9	(1) 42.0	−2.9
5	(2) 21.8	(1) 23.9	(2) 21.7	−4.3
6	(3) 25.4	(2) 26.0	(3) 23.9	−2.7
Sum	186.4	183.2	162.2	−17.8
Block 2:				
7	(1) 30.4	(3) 29.5	(1) 26.7	−1.9
8	(2) 35.2	(1) 33.5	(2) 28.4	−3.4
9	(3) 30.8	(2) 29.3	(3) 26.4	−1.4
Sum	96.4	92.3	81.5	−6.7
Block 3:				
10	(1) 38.7	(2) 37.4	(1) 34.4	−1.7
11	(2) 25.7	(3) 26.1	(2) 23.4	−3.1
12	(3) 21.4	(1) 22.0	(3) 19.4	−3.2
Sum	85.8	85.5	77.2	−8.0
Grand sum	368.6	361.0	320.9	−32.5

Treatment	Q Values
1	8.7[b]
2	−4.1
3	−4.6
Sum	0.0

[a] $-1.5 = 34.6 - 2(32.3) + 28.5$.
[b] $8.7 = (-1.5 - 2.9 - 1.9 - 1.7) - (-5.8 - 4.3 - 3.4 - 3.2)$.

sequence patterns. The data are shown in Table 2 along with the quantities computed prior to the analysis of variance. The numbers in parentheses indicate the treatments, and the data are average daily pounds of FCM produced during three periods of 5 weeks each.

Noting that $p = 3$, $n = 2r = 2(2)$, $v = 3$, and m_1, m_2, and $m_3 = 6$, 3, and 3 for blocks 1, 2, and 3, respectively, the analysis of variance proceeds as follows:

Correction factor:

$$\frac{(-32.5)^2}{3(2)(2)(3)(2)} = 14.67$$

Total sum of squares:

$$\tfrac{1}{6}\left[(-1.5)^2 + (-0.6)^2 + \ldots + (-3.2)^2\right] - 14.67 = 3.72$$

Block sum of squares:

$$\tfrac{1}{6}\left\{\frac{(-17.8)^2}{6} + \frac{(-6.7)^2}{3} + \frac{(-8.0)^2}{3}\right\} - 14.67 = 0.18$$

Treatment sum of squares:

$$\frac{1}{6(2)(2)(3)}\left[(8.7)^2 + (-4.1)^2 + (-4.6)^2\right] = 1.58$$

Error sum of squares:

$$3.72 - 0.18 - 1.58 = 1.96$$

These results are summarized in Table 3.

TABLE 3

The analysis of variance summary

Variance source	Degrees of freedom	Sum of squares	Mean square	F
Total	11	3.72	0.34[a]	
Blocks	2	0.18	0.09	
Treatments	2	1.58	0.79	2.82
Error	7	1.96	0.28	

[a] Ordinarily a mean square is not computed for the total variation, but the computation was done here because the data are uniformity data.

If all sequences had been assumed to have started together, the blocks sum of squares would not have been computed. The error sum of squares would then have been 2.14 with 9 degrees of freedom, yielding an error mean square of 0.24. The failure to obtain significant block or treatment effects is expected, of course, in uniformity data.

The grand mean is

$$\frac{368.6 + 361.0 + 320.9}{36} = 29.18$$

The mean for treatment 1 thus is

$$29.18 + \frac{8.7}{2(2)(3)} = 29.54$$

and the means for treatments 2 and 3 turn out to be 29.01 and 28.99, respectively. The standard error of a treatment difference is

$$\sqrt{\frac{3(0.28)}{2(2)(3)}} = 0.27$$

The high sensitivity of the switch-back trial is attested by the coefficient of variation in these data. If the data were from an actual experiment the coefficient of variation would be obtained as

$$\frac{100\sqrt{0.28}}{29.18} = 1.8\%$$

on the per cow per period basis. Because no block and treatment effects were really present here, the coefficient of variation may be computed as

$$\frac{100\sqrt{0.34}}{29.18} = 2.0\%$$

Somewhat higher values might be expected in actual experiments.

SUMMARY

To take better advantage of the high sensitivity of switch-back or double-reversal trials commonly used to compare two treatments and which have been extended to permit the comparison of three or more treatments, certain convenient and useful features have been added. Designs are given for 3, 4, 5, 6, 7, and 9 treatments. The statistical analysis is outlined symbolically and is illustrated numerically with uniformity data. Missing value formulas are given.

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HISTOLOGY OF THE PITUITARIES, ADRENALS, OVARIES, AND UTERI OF DAIRY CATTLE ASSOCIATED WITH DIFFERENT REPRODUCTIVE CONDITIONS

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Previous studies of the pituitaries, adrenals, ovaries, and uteri of cattle have indicated a relationship between histological changes in these organs and various conditions of reproduction. Several of the cell types found in the anterior lobe of the pituitary have been found to undergo changes in structure or number in relation to the animal's reproductive condition. Garm *(5)*, in a study of normal dairy cows, described the following cell types: chromophobes, acidophils with a small nucleus, acidophils with a large nucleus, small basophils, large basophils, hyaline basophils, and heterotrophic basophils. In cows with nymphomania he found an increase in frequency of large basophils, chromophobes with a large nucleus, hyaline basophils, and hypertrophic amphophils. Two kinds of beta cells in the central zone of the anterior lobe have been described by Bassett *(1)* and Hall *(8)*, Bassett reporting that the small basophil cells were almost absent from steer pituitaries but were increased in pregnant cows or cows treated with stilbestrol. Gilmore *et al. (7)* have described an acidophil cell that seemed to be related to sexual activity of the animal. Cupps *et al. (4)* reported a hyperplasia of the small beta cells in bulls with fascicular tumors of the adrenal gland.

Histological alterations in the adrenal cortex are associated with changes in the reproductive status of cattle. Weber *et al. (12)* have described the occurrence of extramedullary myelopoiesis in the cortex of the adrenal. Many of the cows used in their study were slaughtered because they were infertile. Garm *(5)* described two conditions in sterile cows associated with changes in the adrenal gland. In the first type, which he called nymphomania, there was an increase in weight of the adrenal and width of the adrenal cortex accompanied by a decrease in urinary excretion of neutral steroids. In cows with adrenal virilism the adrenals were also larger, but the urinary excretion of ketosteroids was greater than normal. Both of these syndromes were accompanied by "cystic ovarian degeneration." Houssay *et al. (10)* have reported an increased incidence of adrenal tumors in castrate rats. Some of these tumors produced estrogenic and androgenic effects. Gassner *et al. (6)* have reported finding an androgenic substance in the adrenal venous blood of cows after injections of ACTH.

Casida and Chapman *(2)* have described the occurrence of ovarian cysts in a herd of Holstein cows. The presence of cysts was determined by palpation of the ovary and by the estrous behavior of the cows. Wiltbank *et al. (13)* have reported the incidence of atretic follicles in cows from different sire lines. They defined an atretic follicle as an unruptured follicle more than 2½ cm. in diameter.

Laing *(11)* describes a temporary lowered fertility in cows, which he calls functional ovarian inactivity. He describes varying degrees of ovarian dysfunc-

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tion ranging from no follicular growth to normal follicular growth, ovulation, and faulty development of the ova after fertilization.

The present study is concerned with the histological changes in these organs as they pertain to dairy cattle exhibiting certain types of reproductive failure.

EXPERIMENTAL PROCEDURE

Organs from 56 cows and heifers (6 Holsteins and 50 Jerseys), ranging in age from 14 months to 15 years, have been studied. The animals were grouped as follows:

Group I. 7 cows—pregnant.

Group II. 37 cows—low fertility, irregular cycles, and histological evidence of degeneration of the adrenal gland.

Group III. A. 5 cows—nymphomania, absence of corpus luteum in the ovary.
B. 2 cows—nymphomania, corpus luteum in the ovary.

Group IV. 2 cows—absence of heat, lymphoid areas in the corpus luteum.

Group V. 2 normal open cows and one heifer with bilateral occlusions on the oviducts. The heifer was grouped with the normal cows because she had a history of normal estrous cycles.

In addition, records of the estrous cycles on 13 normal cows (Group VI) were available for comparison with the cycles of Group II. These animals were selected because they were similar in age and ancestry to the animals in Group II. The cycles used for comparison were those recorded while these animals were being bred to a sterile bull. When bred to a fertile bull, they conceived on first or second service.

The pituitary, adrenal, ovaries, and pieces of the uterus were collected from each cow at the time of slaughter. All tissues were fixed in formol-zenker and processed by the paraffin technique. Sections from the pituitary were stained with acid fuchsin, acid violet, and methyl green according to the Severinghaus method. All the other tissues were stained with hemotoxylin-eosin-azure II.

Cells from the two zones of the pituitary were counted in the following way. An ocular micrometer 5 mm. square was placed in one eyepiece of a binocular microscope, and counts were made of 10 fields selected at random within the zone. If the micrometer enclosed an area containing a large blood vessel, the slide was moved until the blood vessel was not in the field counted. Only cells with nuclei were counted; the small beta cells were divided into granular, vacuolated, and hyaline cells. Cell counts associated with various reproductive conditions are reported in Table 1.

Sections of ovaries and uteri from normal cows collected at different stages of the cycle, as reported by Cole *(3)*, were studied for comparison with the ovaries and uteri collected in this series.

Blood eosinophils and urinary steroid excretion of the cows showing adrenal degeneration are reported elsewhere *(9)*. The relative incidence of extramedul-

TABLE 1

Cell counts of the pituitary

Cow No.	Age	Time of slaughter	Group No.	Basophilic zone						Eosinophilic zone[a]	
				Small beta cells				Large beta cells	Chromophobes	Eosinophils	Chromophobes
				Granular	Vacuolated	Hyaline	Total				
	(yr.)			*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
1121	5	4th day post estrous	V	24.7	0.0	0.0	24.7	4.6	69.8	30.6	68.3
972	4	6th day post estrous	V	34.0	0.0	0.0	34.0	5.0	60.0	40.1	59.2
947	8	21st day proestrum	V	29.8	0.0	0.0	29.8	3.5	66.3	39.4	60.0
921	6	pregnant—78 days	I	17.1	11.1	3.8	32.0	3.3	64.7	52.2	45.6
916	6	pregnant—7 months	I	19.8	8.2	0.3	28.3	5.8	65.0	49.4	48.6
1123	4	nymphomania—absence of corpus luteum	III-A.	36.9	0.0	0.0	36.9	5.9	57.2	41.4	57.0
1100	8	nymphomania—absence of corpus luteum	III-A.	35.5	0.0	0.8	36.3	5.3	58.0	49.4	48.3
919	6	nymphomania—corpus luteum present	III-B.	19.7	0.0	5.9	25.6	11.3	62.7	51.4	47.0
991	3	nymphomania—corpus luteum present	III-B.	6.9	0.0	24.4	31.3	5.1	63.3	40.0	58.2
74	2½	8th day post estrous	II	21.9	—	—	21.9	8.5	66.5	30.4	68.3
18	3	24th day post estrous	II	20.4	0.0	1.4	21.8	4.5	73.3	21.1	77.7
755	15	45th day post estrous	II	16.1	0.0	0.0	16.1	10.7	73.0	19.2	80.0
898	10	no heats in 4 months	II	5.8	0.0	0.0	5.8	2.2	92.0	23.4	76.4
902	11	no heats in 8 months	II	4.7	0.0	0.0	4.7	3.8	91.2	15.7	84.0
6	3	no heats in 7 months	IV	22.1	7.9	0.0	30.0	4.0	66.1	46.1	53.7

[a] Occasional eosinophils were found in the basophilic zone and a few small beta cells were found in the eosinophilic zone. These minor cell types are omitted from the table for the corresponding zones so that some of the cell percentages do not equal 100 in all cases.

TABLE 2

The incidence of extramedullary myelopoiesis in the adrenal cortex of cows in relation to their reproductive performance

Group	No.	Myelopoiesis present	Incidence
			(%)
II—Adrenal degeneration	37	31	83.8
III A and B—nymphomania	7	5	71.5
I and V	10	2	20.0

lary myelopoiesis in the adrenal gland for the different groups is reported in Table 2.

RESULTS

In the normal cows of Groups V and VI the average length of the cycle was 22.5 days with a range of 10 to 42 days. The estrous cycles of eight low-fertility cows from Group II averaged 42.2 days in length with a range of 10 to 118 days. Other animals in this group did not show any cycles for as long as 8 months. The cycles of cows in Group II were irregular whether they were bred or not. The two animals that had lymphoid areas in the corpus luteum had not been in heat for 32 days and 7 months, respectively.

Changes in the pituitary associated with these different reproductive conditions are recorded in Table 1. For descriptive purposes the small beta cells are divided into three types—granular, vacuolated, and hyaline. The granular small beta cell is rounded and contains coarse blue granules (Figure 1). The vacuolated cells have a large vacuole or inclusion that stains a lavender color when fixed and stained as outlined above. The nucleus is pushed to one side of the

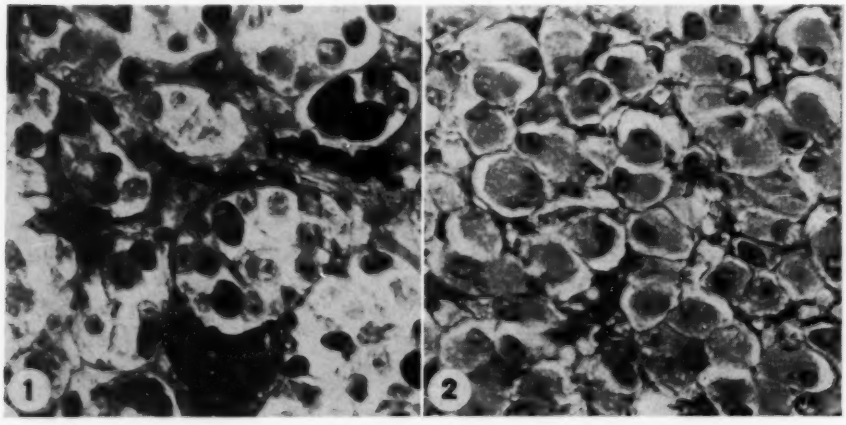

FIG. 1. Pituitary—pregnant cow—small beta cell containing large inclusion in center of picture.

FIG. 2. Adrenal—reticular zone—nymphomania, Group III—B.

cell (Figure 1). The hyaline beta cell contains a pyknotic nucleus and hyaline cytoplasm. In the normal cows the percentage of small beta cells in the central zone of the pituitary ranged from 24.7 to 34.0%. All of the cells were granular. During pregnancy the percentage of granular small beta cells decreased; the percentage of vacuolated and hyaline cells increased; the total number of small beta cells remained within the normal range. In one type of nymphomania the percentage of granular small beta cells increased, whereas in the second type the total percentage of small beta cells was in the normal range, but there was a decided increase in the hyalinized cells. The cows in Group II showed a distinct decrease in the percentages of the small beta cells. Pituitaries from cows in this group with absence of heats for prolonged periods showed the greatest decrease. The cows in Group IV had a normal percentage of small beta cells, but there was a decrease in the granular cells accompanied by an increase in the vacuolated type; these changes are similar to those seen in pregnant cows.

No distinct trends in the large beta cells were found in this study. The percentage of eosinophil cells was decreased in some of the cows showing irregular cycles.

Histologically, the adrenal in the normal cows was similar to that described for the normal bull by Cupps *et al. (4)*. In cows of Group II the adrenal showed histological evidence of degeneration (Figure 3). There was a degranulation of cells in the fascicular and reticular zones. The cells were smaller than those in normal animals and many of the nuclei were pyknotic. The incidence of extramedullary myelopoiesis in animals from Group II is recorded in Table 2. In these animals the areas of myelopoiesis were usually found in the reticular zone. In one type of nymphomania (Group III A, absence of corpora lutea) the cortical cells, especially in the fascicular zone, were hypertrophied and degranulated. The division of fasciculata and reticularis was not distinct. Other cows with nymphomania (Group III B, whose ovaries contained corpora lutea) had a nar-

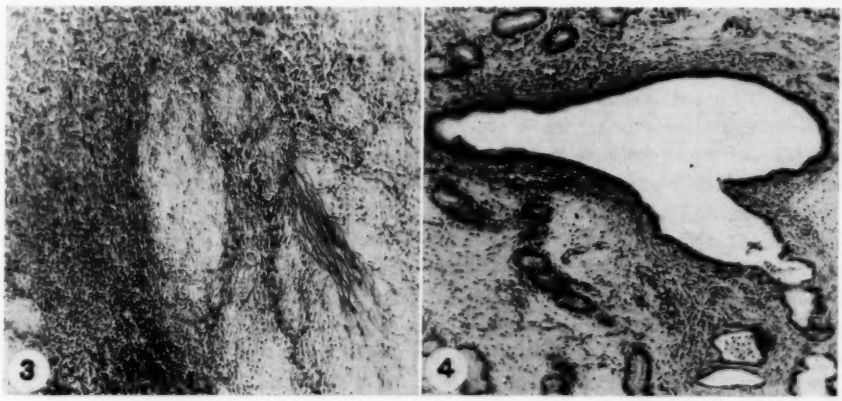

FIG. 3. Adrenal—degeneration of fascicular zone with myelopoiesis.
FIG. 4. Uterine gland with large lumen.

row fasciculata and a hypertrophy of the cells of the reticularis (Figure 2). Myelopoiesis was found in both types of nymphomania. It was usually found in the glomerulosa or at the junction of the glomerular and fascicular layers. During pregnancy there was a hypertrophy of the cortical cells in the adrenal with an abundance of eosinophilic granules in the cytoplasm.

The ovaries from cows of Group III A, which contained no corpora lutea, were large and contained several follicles ranging from 0.8 to 2.5 cm. in diameter. The largest follicles were devoid of granulosa cells, and the thecal cells were swollen and showed degenerative changes. The medium-sized follicles contained both layers and resembled those seen in the normal cows during the proestrum and estrus. The ovaries from cows of Group III B contained corpora lutea and atretic follicles.

Follicular atresia was marked in cows of Group II. The granulosa cells of the ripening follicles from cows of this group did not differentiate and grow to the extent seen in normal cows at the same stage of the cycle. In the normal animals during proestrum and estrus the granulosa was 8 to 10 cells thick. The cells were large with well-defined cell walls, an intermediate amount of cytoplasm, and vesicular nuclei. Follicles from the animals of Group II showed fragmentation of the glomerulosa and a lack of growth and differentiation of the individual cells. Some of the animals of this group had corpora lutea with large central cavities, but not enough control animals were available to compare the incidence of this characteristic in the two groups.

In cows with nymphomania the uterus was enlarged, the endometrium was edematous, and the vascular layer in the myometrium was highly developed. The endometrium of the uterus of the cows in Group II was thinner than that seen in normal cows; furthermore, in some cows the cavities of the uterine glands were enlarged (Figure 4) and surrounded by a thickened spiral of connective tissue.

SUMMARY

Histological studies of the pituitaries, adrenals, ovaries, and uteri of cows exhibiting different types of reproductive failure indicate that some types of low fertility are associated with changes in these organs. In one type associated with irregular estrous cycles or absence of cycles, there is a decrease in the percentage of small beta cells and alpha cells in the pituitary. Changes in the adrenal gland consisted of a narrow fasciculata, a shrinkage of cells of the reticularis, and a high incidence of extramedullary myelopoiesis in the reticular zone of the gland. The ovaries are characterized by a high incidence of follicular atresia with faulty development of the follicle and corpus luteum. The endometrium of the uterus was thin, and in some cases the endometrial glands were enlarged and surrounded by a dense connective tissue.

Histologically, nymphomania was associated with two distinct conditions in the endocrine organs. One appeared to be associated with an increase in the small beta cells of the pituitary, a hypertrophy of the fascicular zone of the adrenal, large numbers of follicles, and the absence of corpora lutea in the

ovaries. The second type was characterized by hyaline degeneration of many of the small beta cells of the pituitary. The cells of the reticular zone of the adrenal were hypertrophied and the ovaries contained corpora lutea and lacked the large number of large follicles seen in the first type. In cows with nymphomania, the myelopoietic areas of the adrenal were seen more often in the glomerular zone.

In two cows that showed no heat periods, the corpora lutea contained discrete areas of lymphoid cells, and the small beta cells were vacuolated or contained large inclusions similar to those seen in pregnant cows. The other organs from these animals appeared to be normal.

During pregnancy, some of the small beta cells of the pituitary contained large inclusions or vacuoles.

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FLUORIMETRIC ESTIMATION OF ESTROGENS IN BOVINE URINE[1,2]

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It is generally agreed that the estrogenic activity of cow urine is low *(7)* compared with the mare and human. Turner *et al. (34)* reported 11 rat units of estrogen per liter in unhydrolyzed nonpregnant cow urine, whereas Nibler and Turner *(19)* found none in one sample. Asdell *et al. (2)*, using bioassay, failed to find estrogen in urine of heifers in heat.

Estrogenic activity of cow urine is low during the first hundred days of pregnancy *(1, 16, 19, 29, 34)* and increases steadily from this time until the end of the gestation period. Daily excretion rates varied from 500 rat units *(19)* to 6,000 rat units *(16)* when urine was extracted without hydrolysis.

Peak excretion rates of 17,000 mouse units per liter *(4)* have been reported for hydrolyzed urine. Turner *et al. (34)* reported that Holstein-Friesian cows excrete more estrogen than Jerseys and that dairy breeds excrete more than beef breeds. Fecal excretion of estrogen, like the urinary excretion, increases markedly during pregnancy *(15, 18)*.

More information on estrogen excretion of dairy cows is highly desirable. The urinary avenue of excretion was selected for this study because it seemed to offer the best chance for success. The purpose of this study was to determine the feasibility of using a chemical method to estimate urinary estrogens and to describe changes in excretion during gestation and estrus.

MATERIALS AND METHODS

Twenty-four-hour urine samples were collected from six milking cows at 50, 100, 150, 200, 250, and 275 days of gestation and once after parturition. One ovariectomized cow also was available for study.

The urine samples were collected by means of a modification of the device described by Hobbs *et al. (17)*. To reduce bacterial contamination, butanol was added *(32)* at a rate of 10 ml. per liter of urine expected. At the end of each 24-hour collection period, the total quantity of urine voided was measured and a 1-l. aliquot was refrigerated until extraction, which was usually within 48 hours.

The chemical method used was a modification of the method of Stimmel *(32)* and of Friedgood *et al. (12)* and consisted of the following steps:

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[1] Scientific Paper No. 1391, Washington Agricultural Experiment Stations, Pullman. Project 1105.

[2] The data are from a thesis presented by the senior author to the graduate faculty of the State College of Washington in partial fulfillment of the requirements for the Ph.D. degree.

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a) A 1-l. aliquot was acidified with concentrated sulfuric acid, with congo red as an indicator, and extracted four times with 0.125 volume butanol.

b) The four butanol extracts were pooled in a separatory funnel and allowed to clarify.

c) The mixed pooled extracts were distilled nearly to dryness in a vacuum still, keeping the temperature of the solution under 100° C. by means of a Glas-col heater. One hundred ml. of distilled water was added and the water extract mixture was distilled again.

d) Thirty ml. of 95% ethanol, 250 ml. of distilled water, and 50 ml. of sulfuric acid were added, and the solution hydrolyzed for 10 minutes at 100° C.

e) Redistilled washed (with 1% ferrous sulfate) ether (100 ml.) was added.

f) After transfer to a separatory funnel, four successive extractions were made with 100 ml. of ether, each time rinsing the flask in which the hydrolysis took place.

g) The ether extract was washed with 20 ml. aliquots of 9% sodium bicarbonate until the aqueous phase became almost clear; then followed three washings with 20 ml. of distilled water.

h) The aqueous phase was discarded and the ether extract transferred to a distilling flask and evaporated to dryness at a temperature under 100° C.

i) Six ml. of ether and 108 ml. of carbon tetrachloride were added. The solution was transferred to a separatory funnel and extracted four times with 50 ml. of 1*N* aqueous potassium hydroxide.

j) The ether phase, containing the neutral steroids, was washed twice with 5-ml. increments of water, and the water wash was then added to the potassium hydroxide (aqueous) phase. The aqueous phase contained the estrogenic phenols. This phase was back-washed with 20 ml. of ether and the ether wash was added to the ether phase. This ether phase contained the neutral steroids which were not analyzed.

k) The aqueous phase was acidified with 6*N* sulfuric acid with congo red as an indicator and then extracted five times with 100 ml. of ether.

l) The ether phase was washed twice with 30 ml. of 9% sodium bicarbonate, twice with 30 ml. of distilled water, and as many times as was necessary with 20 ml. of 2.5% sodium carbonate to obtain a clear solution. The combined sodium carbonate extracts were back-washed with 100 ml. of ether, and the ether wash was added to the ether phase. This was then washed twice with 20 ml. of distilled water.

m) The ether phase was distilled to dryness and the residue taken up in 10 ml. of 95% ethanol and 40 ml. of distilled water and steam distilled until 500 ml. of distillate was obtained. A volume of about 50 ml. was maintained by means of a Glas-col heater.

n) After the steam distilled ether solution containing the phenolic steroids was evaporated to dryness, the dried residue was taken up in 10 ml. of 95% ethanol.

o) After a 1-ml. aliquot of the alcoholic solution was again evaporated to dryness, 10 ml. of 85% phosphoric acid was added. The solution was heated in a boiling water bath for 30 minutes and then allowed to cool.

p) The fluorescence was measured in a Coleman Model 12 photofluorometer with a lamp filter transmitting at a wave length of 436 mμ (accomplished by Corning #3385 and Corning #5113 glass filters) and photo cell filter transmitting at 525 mμ (accomplished by a Baird 525 mμ interference filter and a Corning #3385 glass filter). The fluorescence of the unknown sample was compared with the fluorescence of a standard of crystalline estrone. This standard was also used for bioassay comparisons.

Bioassay determinations were made on some of the samples by means of the 6-hour uterine weight response method of Astwood, modified as suggested by Emmens *(9)*, to a four-point assay based on a crude uterine weight response with covariance for body weight corrections. Estrone served as the standard with 0.1, 1, 2, 4, and 8 γ in aqueous solution yielding a linear log dose relationship with increase in crude uterine weight. The 0.1 and 1.0 γ standard was used for each four-point assay employing 20 rats with five in each group. Injections were made subcutaneously. Owing to the small number of rats per group the assays, while qualitative, were only approximate quantitatively.

The chromatographic separation technique described by Woods *(37)* was also used. Briefly the procedure was:

A 19 mm. (inner diameter) × 200 mm. interjoint chromatograph tube was used for fractionation of the estrogens on alumina. The column was prepared by making a thin slurry of alumina in benzene and pouring it rapidly into the tube. The tube was packed by tapping gently while under slight vacuum to a height of 150 ± 2 mm. when most of the benzene had been removed. Glass wool was placed on top of the column after removal of vacuum. The column was not permitted to dry.

Thoroughly dried residue containing phenolic steroids was dissolved in 20 ml. of 1.5% methanol in benzene and added to the column. The flask was rinsed with 20 ml. of benzene, in small portions, and the washings were added to the column until the meniscus of the solution was within 0.5 cm. of the top of the column. Fractions corresponding to estrone, estradiol, and estriol were collected from the following eluents with successive 200 ml. portions of 1.5, 5.9, and 50.0% methanol benzene, respectively.

RESULTS

Evaluation of the methods. Three recovery trials with 10 γ of added estrone per liter of urine resulted in an average recovery of 94.6% of the added estrone (range 93 to 96%).

Chromatographic separation. Trials were made with crystalline estrone, estradiol, and estriol. The results as shown in Table 1 reveal overlapping of the fractions. On the average, 55.5% of the estrone, 59.0% of the estradiol, and 66.8% of the estriol were recovered in their respective eluates. The average loss

TABLE 1

Results from adding 100 γ of estrone, estradiol, and estriol separately to a chromatograph column of alumina

Eluate	50 ml/fraction	Estrone	Estradiol	Estriol
	(No.)	*(γ)*	*(γ)*	*(γ)*
1.5% methanol in benzene	1	1.5	0.0	0.0
	2	12.5	0.0	0.0
	3	31.2	0.2	0.0
	4	10.3	1.9	0.0
	Total	55.5	2.1	0.0
5.9% methanol in benzene	5	7.1	5.0	0.0
	6	5.3	17.1	0.5
	7	2.1	24.0	1.7
	8	0.0	12.9	3.2
	Total	14.5	59.0	5.4
50.0% methanol in benzene	9	0.0	4.8	13.9
	10	0.0	3.2	33.8
	11	0.0	0.0	14.3
	12	0.0	0.0	4.8
	Total	0.0	8.0	66.8
No. of trials averaged		6	7	3
Per cent total recovery		70.0	69.1	72.2

for all three fractions was 29.6%. Some of this loss may have been due to pipetting inaccuracies. It was more likely due to estrogens remaining on the alumnina column.

Urine extracts from six cows were chromatographed. As shown in Table 2, the primary estrogen in cow urine appears to be estradiol. This is in agreement with the results of Woods *(37)*.

Quantities of estrone, estradiol, and estriol found in their respective eluates were quite constant for pregnant and nonpregnant cows. These values are probably 30% or more too low because of inefficiency of the column (Table 1). The cow carrying the mummified fetus showed more estrone than the other cows.

TABLE 2

Chromatographic separation of estrone, estradiol, and estriol from urine

Cow	Physiological state	Total fluorescence-producing compounds in estrone equivalents	Estrone	Estradiol	Estriol
(No.)		*(γ/liter)*	*(γ)*	*(γ)*	*(γ)*
2147[a]	250 days gestation	253	32.0	54.0	7.2
316	275 days gestation	690	4.6	77.6	5.8
3070[b]	200 days gestation	400	2.4	61.5	5.0
3070[c]	2 days post estrum	231	2.0	65.5	7.3
3060[d]	1 day post estrum	188	2.4	61.5	8.9
3060	13 days post estrum	165	2.0	65.5	6.9

[a] Delivered mummified fetus 42 days later.
[b] Bioassay value was 111 γ/liter of urine (Table 3).
[c] 28-day nonservice cycle.
[d] 23-day nonservice cycle.

TABLE 3

Comparison of chemical and bioassay estimation of urinary estrogen (estrone equivalents per liter of urine)

Cow	Stage of gestation	Bioassay	Total chemical
(No.)	*(Days)*	*(γ)*	*(γ)*
2155	150	30	330
2155	200	119	340
3902	200	87	370
3902	275	143	500
3070	200	111	400
2147[a]	150	137	360
359[b]	—	5	20

[a] Mummified fetus.
[b] Ovariectomized.

Total fluorescence-producing compounds were higher for pregnant cows (cow 2147 not normal) than for nonpregnant cows.

Other data by Smith *(28)* have shown total fluorescence in estrone equivalents averaging 194 ± 19, 224 ± 23, and 245 ± 50 γ per liter of urine, respectively, for day of estrus and 7 and 14 days post-estrus (average for five cows).

Chemical and bioassay methods comparison. This comparison is shown in Table 3. Although the correlation of the two methods was high (+0.81; $P > 0.05$), the chemical results averaged 3.7 times as high. Also, cow 359 that was ovariectomized at 26 months of age and 10 months before urine assay started showed 5 γ of estrone equivalent per liter of urine by bioassay (55 γ in 24 hours)

TABLE 4

Fluorescence-producing compounds excreted during the gestation period

Cow	Days of gestation						Post-parturient	
	50	100	150	200	250	275	Value	Days
(No.)	*(γ estrone equivalent/liter of urine)*							
339	38	77	360	380	430	420	231	5
316	58	250	300	350	680	690	229	17
2155	56	290	330	340	550	1700	590	12
2147	51	157	360	380	253	125	47	4
3902	35	290	330	370	420	500	—	—
3070	91	230	350	400	420	410	160	10
	(γ estrone equivalent/24 hour period)							
339	513	978	3960	5320	7310	5880	3234	5
316	870	5000	6900	8225	13600	13800	3778	17
2155	448	2465	2805	3400	5390	10200	3540	12
2147	434	1256	2880	4940	1771	875	306	4
3902	480	3915	3465	3996	6300	7000	—	—
3070	637	2898	4900	5400	5250	5740	2240	10
	(γ estrone equivalent/100 lb. live weight)							
339	33.9	64.7	261.9	351.9	483.5	388.9	213.8	5
316	56.4	324.3	447.5	533.4	882.0	894.9	245.0	17
2155	41.6[1]	228.9	260.4	315.6	500.5	947.1	328.7	12
2147	43.2	125.1	286.9	492.0	176.3	87.1	29.4	4
3902	49.1	400.7	354.7	409.0	644.8	716.5	—	—
3070	49.2	223.8	378.4	416.4	405.4	443.2	173.0	10

and 20 γ for the chemical determination (210 γ in 24 hours). Five other chemical assays on this cow throughout the experimental period yielded excretions of 25 to 32 γ of estrone equivalents per liter of urine. The ovariectomized cow showed chemical values well below those observed for intact cows, thus indicating that the fluorescence-producing materials are associated with ovaries and placenta.

Changes in excretion rates during gestation. Table 4 reveals considerable between-cow variation in total fluorescence-producing compounds. However, all cows (except 2147 with mummified fetus) showed steady increase from the 50th day of gestation until near parturition. Decreases were observed after parturition. These findings are in general agreement with previous bioassay results *(1, 4, 18, 19, 31)*. Cow 2147 delivered a mummified fetus on the 295th day of gestation. This cow showed an apparently normal excretion rate at 200 days but was considerably lower at 250 days and much lower than the other cows 4 days after parturition. This suggests a change in placental function some time between 200 and 250 days in gestation *(1, 19)*. See Table 5 for description of cows used.

TABLE 5
Description of cows used

Cow	Breed	Age of calving	Type of birth	Length of gestation	Parturition to first estrus	Reproductive history
(No.)		*(Yr.)*		*(Days)*	*(Days)*	
339	Hol.	4	male	288	47	Normal
316	Hol.	5½	female	283	74	Normal
2155	Jer.	5½	male	277	13	Normal
2147	Jer.	3⅓	mummy[a]	295	5	Previously normal
3902	Guer.	8¼	female	290	—	Previously normal, this calf and cow died
3070	Guer.	4	male	283	11	Normal

[a] Stilbestrol forced delivery.

DISCUSSION

The chemical method used in these investigations revealed variations in the amounts of fluorescence-producing compounds with advancing pregnancy in dairy cattle. The extent to which estrogens contributed to this variation is not known with certainty in spite of the fact that the method is capable of extracting and estimating with considerable accuracy estrone added to urine. The experiments with the chromatographic column indicated that considerable fluorescence in urine extracts from pregnant cows was not due to estrone, estradiol, and estriol, even though it cannot be asumed that the column used separated these completely or specifically from the crude extracts. In this latter regard it seems somewhat strange that the values obtained after chromatography should be so similar for cycling and pregnant cows. The correlation of the chemical values with bioassay values (+0.81) in a limited number of trials suggests that the variation observed may have been due to variation in estrogen content. The low values obtained by bioassay as compared to those obtained by the chemical

method indicate also that considerable nonspecific fluorescence contributed to the values obtained in spite of the fact that bioassay values have their limitations when derived from injection of crude extracts. The exact significance of the variation in fluorescence-producing compounds during pregnancy therefore awaits further description of the nature of the compounds contributing to the fluorescence.

Further experiments on hydrolysis, extraction, and purification procedures are needed before a satisfactory chemical procedure for urinary estrogens can be developed. Because hydrolysis procedures were necessary *(12, 13, 21, 24, 30, 31, 37)* and because estrogens are readily oxidized *(24, 35, 36)*, it is likely that not all estrogens originally present in the urine were measured. It is also likely that other fluorescence-producing compounds are produced, as indicated in Table 2 and as previously reported *(24)*. These could, in part, be unknown metabolites of estrogens that were originally present in the animal in a biologically active form.

Another troublesome feature of the chemical method used is that a brown colored impurity remains *(37)*. Though the effect of this colored material on total fluorescence may not be serious *(5)*, efforts continue to more clearly delineate members of the group of phenolic steroids *(3)*. Chemical procedures may result in values too high because of nonestrogenic fluorescence-producing materials but may be too low because of *(a)* oxidation, *(b)* incomplete hydrolysis of conjugates, *(c)* interfering impurities, *(d)* transfer losses and *(e)* variation in intensity of fluorescence for the various types of estrogens.

Bioassay likewise has serious limitations in addition to species and method differences in response. These include augmentation effects of impurities *(23, 24)* and ability to measure only the estrogens excreted in a biologically active form, which in turn give variable biological activities depending on the types of estrogen present *(11, 22, 24)*. When purification and separation of the component compounds is resorted to, the bioassay estimates are subject to the same criticism as chemical endpoints for estimation.

Evidence is good that biologically active estrogens are transformed into other products by the uterus *(8, 15, 20, 25)* and liver *(6, 8, 10, 14, 15, 20, 25, 26, 27, 33)*.

Experiences from this study of urinary fluorescence-producing compounds show that the chemical method is faster and less costly compared with bioassay but is still time-consuming and exacting. Strict adherence to procedures should result in error in the same direction, thereby making it possible to compare excretion rates for various reproductive conditions whether normal or abnormal. This is an applied concept which should not be confused with the more fundamental problems of identification, biological action, and identification of the phenolic steroids.

SUMMARY

A chemical method which is capable of extracting and estimating estrogen in the urine of cattle has been described. When applied to the urine of pregnant

cattle, the total fluorescence given by this method increased with advance in pregnancy. The extent to which estrogenic compounds contributed to this variation has not been accurately determined over the whole range of values obtained. Bioassay values on a limited number of samples indicated that about one-fourth of the fluorescence was due to biologically active estrogens.

Further work on extraction and purification of the urine extracts and identification of the compounds contributing to the fluorescence is necessary to develop a satisfactory method for estrogens in cow urine.

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A HIGH ROUGHAGE SYSTEM FOR RAISING CALVES BASED ON EARLY DEVELOPMENT OF RUMEN FUNCTION. VI. INFLUENCE OF HAY TO GRAIN RATIO ON CALF PERFORMANCE, RUMEN DEVELOPMENT, AND CERTAIN BLOOD CHANGES

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Early establishment of mature type rumen function encouraged in young calves by feeding rations high in roughage along with fresh cud inoculations has been shown to be an aid in producing low cost dairy herd replacements *(5, 6, 7, 15, 16)*.

In this experiment a study was made of the effects of feeding three different ratios of hay to grain (4:1, 3:2, 2:3) and an exclusive milk diet on feed consumption, growth, and efficiency of feed utilization. Also measured were changes with age in rumen volatile fatty acids, rumen pH, rumen microorganisms, blood glucose, and blood cholesterol in calves fed the various rations.

EXPERIMENTAL PROCEDURE

Twelve Jersey and nine Holstein calves were assigned to one of three groups at birth to be fed either a 4:1, 3:2, or 2:3 ratio of hay to grain. After a 3-day colostrum feeding period whole milk was fed twice daily at the daily rate of 1 lb. per 10 lb. of birth weight. No milk was fed after 7 weeks.

Beginning on the fourth day and continuing to 12 weeks of age, chopped, good quality, mixed clover-timothy hay (11.5% protein) was offered free choice. In order to obtain the hay to grain ratio desired a simple (18% protein) grain mixture was fed, the amount being regulated by the quantity of hay being consumed.

Fresh warm cud inoculations taken from older cattle in the herd were given weekly for the first 6 weeks. Salt, water, and steamed bonemeal were provided free choice in each pen.

For purposes of comparison with calves fed hay and grain, four Jersey calves were fed exclusively on whole milk to 12 weeks of age at the daily rate of approximately 1 lb. per 10 lb. of body weight. These calves were maintained without bedding, with Neomat pads on the pen floor.

Records were kept of daily feed consumption, weekly body weight changes, and withers height at birth and at 12 weeks of age. Whole blood glucose, determined by the method of Somogyi *(20)*, and plasma cholesterol, determined by the method of Saifer and Kammerer *(18)*, were measured at weekly intervals throughout the 12-week experimental period.

As an index of the effect of the various ratios of hay to grain on developing rumen function, determinations of the pH and total steam volatile fatty acid

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content of strained rumen juice, obtained by stomach tube, were made at 4, 6, 9, and 12 weeks of age. Information on the establishment of several indicator rumen microorganisms was obtained with wet preparations and Gram stains of the same rumen juice samples, according to rating methods previously described *(15)*.

Results of studies made in conjunction with this experiment on rumen riboflavin and thiamine synthesis and digestibility of dry matter, cellulose, and protein have been reported previously *(1, 2)*.

Feed consumption, utilization, and growth. The average feed consumption, the efficiency of feed utilization calculated as TDN per pound of gain, and the gain in body weight and height at withers during the first 12 weeks of the calves fed the three hay to grain ratios (4:1, 3:2, and 2:3) are shown in Table 1. The relatively poor average performance of the calves fed the 3:2 hay to grain ratio was apparently due to delayed consumption of dry feed by certain calves.

The feed consumption and performance of calves fed exclusively on milk to 12 weeks of age are also indicated in Table 1. Of special interest was the high efficiency of milk TDN compared to the other rations. That the balance of nutrients in milk appeared to be superior to the other rations for skeletal growth was indicated by the observation that the gain in withers height of the all-milk fed calves was greater, although their body weight gains were no more, and their TDN intake was less, than those of the calves fed the 2:3 hay to grain ratio.

In order to show more definitely the effects of the three hay to grain ratios uncomplicated by milk feeding, the above data are also shown for the period 8-12 weeks of age in Table 1. It will be noted that weight gains, TDN intake, and digestible protein increased as the grain feeding in relation to hay was increased. Corresponding increases in efficiency of feed utilization were also indicated by TDN per pound of gain. It should be pointed out that these apparent advantages, in so far as herd replacements are concerned, are of questionable economic value when it is considered that the extra gains are not marketed. Furthermore, the extra gains at 12 weeks due to the heavier grain feeding are later lost when greater dependence is placed upon roughage consumption.

It is likely that the increased gains, as well as the higher nitrogen retention, reported previously *(1)* were principally due to the higher level of energy intake as the amount of grain in relation to hay increased. However, when the gains in weight were adjusted for differences in TDN intake in Table 1 by using the regression of gain on TDN intake in the analysis of covariance *(19)*, significant differences ($P < 0.05$) still remained which could not be accounted for on the basis of TDN intake. The differences remaining among the adjusted mean gains may have been caused by the increase in digested protein at the higher levels of grain feeding. The consistently high efficiency of protein utilization at all three ratios measured as digested protein per pound of gain supports this reasoning and suggests that protein may have been a limiting factor in the growth of the calves receiving the 4:1 and 3:2 hay grain ratios.

TABLE 1

The performance of dairy calves fed 4:1, 3:2, and 2:3 ratios of hay to grain or only milk to 12 weeks of age

	4:1 Ratio		3:2 Ratio		2:3 Ratio		Milk only
Breed	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey
No. of calves	4	3	4	3	4	3	4
Whole milk *(lb.)*	218	379	214	380	212	359	613
Grain, 12 wk. *(lb.)*	20.7	36.1	32.6	69.2	84.7	105.6	———
Hay, 12 wk. *(lb.)*	91.2	151.6	58.2	141.3	68.4	77.9	———
TDN, 12 wk. *(lb.)*[a]	99.0	168.8	91.6	191.3	139.7	185.8	99.3
Birth wt. *(lb.)*	54.5	92.0	53.0	96.0	58.0	89.0	56.0
Gain in wt., 12 wk. *(lb.)*	50.0	85.0	45.0	85.0	64.0	93.0	64.0
Gain in withers ht., 12 wk. *(in.)*	3.00	4.58	2.94	4.42	4.44	4.25	5.63
TDN/lb gain, 12 wk. *(lb.)*[a]	1.98	1.97	2.03	2.25	2.18	2.00	1.55
Gain in wt., 8-12 wk. *(lb.)*[b]	20.8	30.3	21.0	44.0	32.3	56.0	33.0
TDN, 8-12 wk. *(lb.)*[a]	49.7	73.8	45.6	88.4	65.6	94.9	45.3
TDN/lb gain, 8-12 wk. *(lb.)*[a]	2.40	2.44	2.17	2.01	2.03	1.69	1.37
Adjusted mean gain 8-12 wk. *(lb.)*[c]	23.6	37.1	25.8	43.5	27.7	52.9	———
Dig. protein (apparent), 8-12 wk. *(lb.)*	6.39	9.49	6.06	11.76	9.66	13.97	11.38
Dig. protein/lb gain, 8-12 wk. *(lb.)*	0.30	0.31	0.29	0.27	0.30	0.25	0.35

[a] TDN calculated from Morrison's *(12)* appendix, Table 1.

[b] Least significant difference between any two group means = 9.7 lb.

[c] Mean gain (adjusted for TDN) = y-bx, where y = group mean gain, b = regression coefficient and x = deviation from the total mean (Snedecor, *19*).

These observations indicate the need of further work to determine the proper balance between protein and energy sources in both roughages and concentrates when fed in different ratios and combinations for most efficient use of both.

Rumen development. The volatile fatty acid content of rumen juice increased from 56-64 meq. per liter in the initial samples at 4 weeks of age to a maximum of 84-99 meq. at 9 weeks of age (Table 2). That the pH was increasing during this period is shown in Table 3, suggesting that buffering capacity increased as

TABLE 2

Effect of age and ratio of hay to grain fed on the total steam volatile fatty acid content of rumen juice

Age range	No. of calves	Total steam volatile fatty acids		
		4:1 Ratio	3:2 Ratio	2:3 Ratio
(wk.)		*(Av. meq/l)*	*(Av. meq/l)*	*(Av. meq/l)*
4-5	7	56.3 ± 8.1	59.2 ± 3.9	63.5 ± 6.9
6-7	7	69.0 ± 5.4	70.0 ± 6.8	74.7 ± 2.7
9-10	7	84.1 ± 4.8	99.2 ± 5.0	94.8 ± 7.6
11-12	7	86.1 ± 3.2	98.3 ± 5.4	90.7 ± 3.1

TABLE 3

Effect of age and the ratio of hay to grain fed on the pH of rumen juice

Age range	pH					
	4:1 Ratio		3:2 Ratio		2:3 Ratio	
	Average[a]	Range	Average	Range	Average	Range
(wk.)						
4-5	6.42 (4)[b]	6.10-6.83	6.28 (6)	6.12-6.52	6.14 (4)	5.81-6.69
6-7	6.64 (5)	6.22-6.80	6.01 (6)	5.68-6.60	6.33 (6)	6.20-6.50
9-10	6.73 (7)	6.51-7.00	6.31 (7)	5.90-6.80	6.37 (7)	5.90-7.20
11-12	6.76 (7)	6.61-6.95	6.50 (5)	6.30-6.88	6.62 (6)	6.39-6.93

[a] pH calculated from average hydrogen-ion concentration.
[b] Figures in parentheses indicate number of calves represented in the average.

the hay intake increased. Statistical analysis of the data showed highly significant differences between 4 and 12 weeks of age for both total volatile fatty acids and hydrogen-ion concentration of rumen juice. The ratio of hay to grain fed did not significantly affect the level of total volatile fatty acids in the rumen juice.

Differences in the hydrogen-ion concentration between the 3:2 or 2:3 ratio and the 4:1 ratio at 4, 6, and 9 weeks of age were highly significant (Table 3). Differences in pH of rumen juice at 12 weeks were not statistically significant. Low rumen pH associated with high levels of grain feeding has been observed previously *(9, 14)*. An indication of early rumen development in these calves is found in the pH values (Table 3), which were in the range reported for mature cattle fed alfalfa hay *(9)* in contrast to the low pH values of 4.09 to 5.80 reported by Kesler *et al. (8)* in Holstein calves fed hay and a calf starter ration, free choice to 5 lb. daily.

A relatively higher total volatile fatty acid level was previously observed in the rumen juice of calves fed alfalfa hay at these same three hay to grain ratios *(3)*. Since the volatile fatty acids measured in rumen juice represent the quanti-

ties not yet absorbed from the rumen and since their rate and mechanism of absorption has been found to be regulated by rumen pH *(4)*, the higher levels found when alfalfa hay was fed probably resulted from a combination of the effects of the higher content of alkaline ash on rumen pH and the higher rate of fermentation shown to be supported by alfalfa hay *(10)*.

The establishment of indicator rumen microorganisms coincided in general with pH increases, as shown in Tables 3 and 4. This further emphasizes the importance of early hay consumption as a rumen pH regulator in the early establishment of mature type rumen function.

TABLE 4

Effect of age and the ratio of hay to grain fed on the establishment of indicator rumen microorganisms[a]

Ratio of hay to grain fed	Indicator micro-organisms rated[a]	Average rating			
		4-5 wk.	6-7 wk.	9-10 wk.	11-12 wk.
4:1	Protozoa[b]	1.80 (5)[e]	2.67 (6)	3.33 (6)	3.57 (7)
	Hay I[c]	3.20 (5)	2.33 (4)	3.00 (7)	2.00 (6)
	Hay II[d]	3.00 (5)	3.33 (4)	3.57 (7)	5.00 (6)
3:2	Protozoa	1.00 (6)	2.33 (6)	3.00 (6)	3.80 (5)
	Hay I	2.00 (6)	2.75 (4)	3.14 (7)	3.00 (6)
	Hay II	0.50 (6)	2.00 (4)	2.29 (7)	3.33 (6)
2:3	Protozoa	2.12 (6)	3.00 (5)	3.00 (6)	4.00 (6)
	Hay I	3.18 (6)	2.33 (6)	3.00 (7)	2.83 (6)
	Hay II	0.83 (6)	0.50 (6)	1.29 (7)	1.16 (6)

[a] Indicator microorganisms and method as previously described *(15)*.
[b] Protozoa rated 0-4 using wet preparations.
[c] Hay I—one bacterium rated 0-4, using Gram's stain.
[d] Hay II—three bacteria rated individually 0-4 and summed, using Gram's stain.
[e] Figures in parentheses indicate number of calves represented in the average.

As the rate of grain feeding was increased from the 4:1 ratio to the 2:3 ratio, a marked reduction in ratings of the Hay II bacteria was observed (Table 4). The level of grain feeding in this experiment did not appear to markedly influence the ratings of either protozoa or the Hay I bacteria. These observations are in agreement with previous findings *(6, 15)*.

Thus it seems advantageous in raising herd replacements to sacrifice some gains in body weight during the early months in order to obtain early mature type rumen function and thus take advantage of the economy of high roughage feeding.

Blood changes. Blood glucose was found to range from 80-87 mg. % at 1 week of age, 52-58 mg. % at 8 weeks of age after milk feeding was discontinued, and approximately 60 mg. % at 12 weeks, with no marked differences due to the various ratios of hay to grain fed (Figure 1). A sharp decline in blood glucose occurred between 6 and 8 weeks of age, which was apparently associated with reduction in milk feeding.

A decline in blood glucose during the first 8 weeks of age was reported by Murley *et al. (13)* in skimmilk-fed calves. They interpreted the drop in blood glucose as not being a function of the dietary shift from milk to feeds which

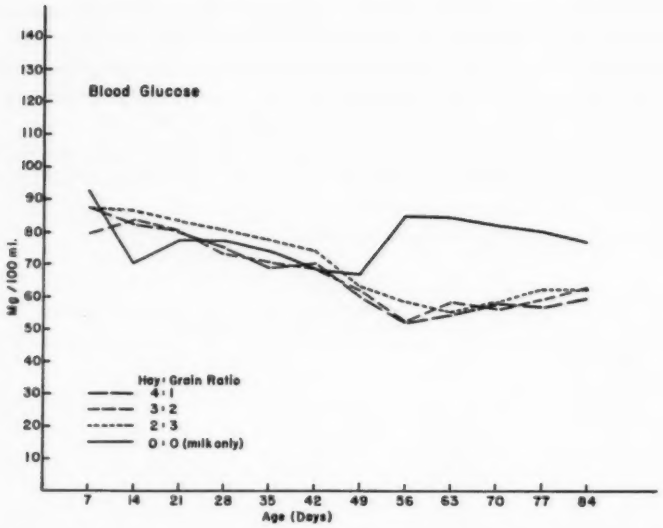

FIG. 1. Changes in blood glucose in calves fed different ratios of hay to grain or an exclusive milk diet.

undergo microbial fermentation in the rumen, as McCandless and Dye have suggested *(11)*. Reid *(17)*, working with lambs, showed that the major portion of the decrease in blood glucose during the first 4 to 5 weeks was due to a decrease in corpuscle glucose. Unpublished results obtained in this laboratory showed that this was also true for calves. Thus, the usual decrease in blood glucose in the first 8 to 9 weeks is probably due to the combination of the drop in corpuscle glucose during the first 4 to 5 weeks and the effects associated with the transition from a simple-stomached animal to a ruminant.

A general downward trend in blood glucose levels occurred also in the calves fed only milk during the first 7 weeks of age, but a higher level of 78-85 mg. % was maintained from 8 to 12 weeks of age. The decline in the exclusively milk-fed calves during the first 7 weeks probably resulted in part from several factors, including the limited milk feeding, diarrhea, and the decline in corpuscle glucose.

Blood cholesterol increased in all groups during the first 3 weeks of age. It remained at a relatively high level until milk was taken out of the ration, which resulted in a decline to approximately the level of 1 week of age (Figure 2). Calves fed milk and no roughage for 12 weeks maintained a high blood cholesterol level until the end of the experiment.

It was noted that wide variations existed in the cholesterol level of individual calves, which tended to persist throughout the experiment. Because of the wide variations among the calves no significance can be attached to differences among the three groups fed different hay to grain ratios.

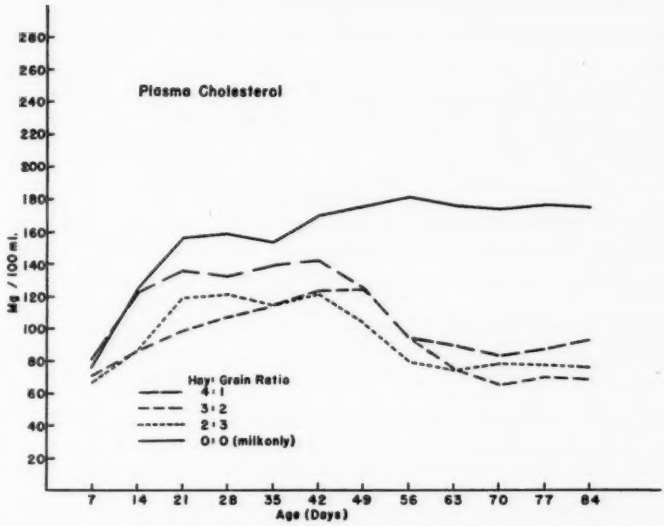

FIG. 2. Changes in blood cholesterol in calves fed different ratios of hay to grain or an exclusive milk diet.

SUMMARY AND CONCLUSIONS

Growth, feed consumption, and efficiency of feed utilization were studied during the first 12 weeks in 21 rumen-inoculated Jersey and Holstein calves fed either 4:1, 3:2, or 2:3 ratios of mixed clover-timothy hay to grain. Observations were also made on rumen development and certain blood changes.

As the amount of grain was increased in proportion to the amount of hay fed, increasingly greater weight gains, TDN intake, efficiency of feed utilization, and per cent of protein digested were observed.

An apparent superiority of the balance of nutrients in milk for skeletal growth was indicated by the larger gain in withers height in the calves fed all milk to 12 weeks of age, although their body weight gains were no more and their TDN intake was less than those of calves fed the 2:3 ratio of hay to grain.

Although total volatile fatty acids increased from 4 weeks of age to a maximum at 9 weeks, no effect of hay to grain ratio on the total volatile fatty acid content of the rumen juice was found. Rumen pH increased with advancing age but was maintained at a lower level in the calves fed the larger amounts of grain.

Rumen bacteria and protozoa ratings gradually increased during the 12-week period. The ratings of the Hay II group of rumen bacteria, however, were reduced drastically as the proportion of grain to hay was increased.

Blood glucose was not markedly influenced by the ratio of hay to grain fed. A high blood glucose level was maintained in calves fed only milk.

After an initial increase during the first 3 weeks, blood cholesterol declined when milk was taken from the ration. Calves fed only milk maintained a high

blood cholesterol level throughout the experiment. Wide variations existed among calves, but no significant differences could be attributed to the ratio of hay to grain fed.

Further work will be required to determine the best balance between grain concentrates and various roughages required to produce satisfactory growth along with optimum rumen function.

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DILUTERS FOR BOVINE SEMEN. VIII. THE EFFECTS OF ALTERATIONS OF SOME PHYSICAL FACTORS OF A MILK DILUTER ON THE LIVABILITY OF BULL SPERMATOZOA[1,2]

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Several workers have shown that a relationship exists between the osmotic pressure and electrolyte concentration of diluters *(1, 4, 5)*. Kampschmidt *et al. (4)* found that partial replacement of the sodium containing buffer salts in an egg yolk–buffer diluter by solutions of metabolizable sugars resulted in increased survival of bull spermatozoa during storage, provided a sufficient quantity of buffer remained to maintain an optimum pH level during storage. In his monograph, *Biochemistry of Semen*, Mann *(5)* has indicated that the ultimate effect of tonicity depends upon certain other prevailing conditions, including the ratio between electrolytes and nonelectrolytes that contribute to the tonicity. Work by Emmens *(1)* with rabbit spermatozoa indicated that pH is also a complicating factor in studies with osmotic pressure and electrolyte concentration.

This paper will report the effects of independent variations in pH, osmotic pressure, and electrolyte concentration in a milk diluter and their interactions on the livability of bull spermatozoa.

EXPERIMENTAL PROCEDURE AND RESULTS

The diluter employed in these studies was composed of reconstituted nonfat dry milk solids detoxified by the addition of cysteine HCl rather than by heating *(3)*. The composition of the diluter was as follows:

8.7 g. Instant Pet nonfat dry milk solids
100 ml. distilled water
100 mg. cysteine HCl
100 mg. streptomycin sulfate

Previous work on the development of this diluter *(2)* has shown that it results in improved livability when compared with heated or cysteine-treated fresh skimmilk. A particular improvement has been noted in the consistency of results obtained among lots of cysteine-treated reconstituted dry diluters when compared with aliquots of the same lots heated at 92° C. for 10 minutes.

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[2] The data contained in this paper are part of a thesis submitted by the senior author to the Graduate School of The Pennsylvania State University in partial fulfillment of the requirements for the degree of Master of Science.

Procedures used in the dilution and storage of semen were the same as previously reported *(3)*. The split ejaculate technique was employed, and 10 or 12 ejaculates were included in each trial. A Beckman model G potentiometer was used to determine pH. Osmotic pressure was determined by means of freezing point depression measurements with a Hortvet cryoscope, which depends upon the evaporation of ether for the cooling effect. The sample was "seeded" when necessary to avoid extremes of super-cooling. The Beckman thermometer was read accurately to 0.01° C., and estimations were made to 0.001° C. by means of a magnifying glass corrected for parallax. Statistical analyses were conducted according to methods outlined by Snedecor *(7)*.

Three preliminary trials were conducted in an effort to choose the two or three most meaningful levels of pH, electrolyte concentration, and osmotic pressure to be used in a factorial experiment. Narrow ranges in variations were chosen in order to give information of a practical nature.

In the first of the three preliminary trials, the desired pH levels of the diluter were obtained by the addition of 0.1 *N* HCl or 0.1 *N* NaOH. Osmotic pressure and electrolyte concentration were maintained constant by the addition of 0.1 *N* NaCl as required. The amounts of added electrolytes and the mean livability

TABLE 1

Alteration of the pH of cysteine-treated nonfat dry milk solids diluter and its effect on the livability of bull spermatozoa

Treatment	pH adjusted to:	Ml. 0.1 *N* electrolyte added per 100 ml. basic diluter				Actual pH attained	Mean livability[a]
		HCl	NaOH	NaCl	Total		
1	6.0	5.8		10.2	16.0	6.2	37.9
2	6.3	1.9		14.1	16.0	6.4	42.7
3	6.6		2.1	13.9	16.0	6.7	43.5
4	6.9		6.1	9.9	16.0	6.9	38.4
5	7.2		9.8	6.2	16.0	7.2	28.7
6	7.5		14.0	2.0	16.0	7.5	22.6
7	7.8		16.0		16.0	7.8	15.1

[a] Sum of mean motility readings of 10 ejaculates for day 1, 2, 3, 4, 6, 8, 10, and 12 divided by 8.

results of ten ejaculates are presented in Table 1. There were distinct treatment differences due to changes in pH. A pH level of 6.7 gave the best results, with poorer results found in more acid and more alkaline media.

In the second of the three preliminary trials, the osmotic pressure and pH were maintained constant and electrolyte concentration was varied. Since osmotic pressure is partly dependent upon electrolyte concentration, it became necessary to find a nonelectrolyte material that would have the same effect on osmotic pressure as an electrolyte. After several trials, an 8% aqueous solution of glycerol by weight was found to increase the freezing point depression of the diluter approximately 0.01° C. when 1.0 ml. was added to 100 ml. of the diluter, as did 0.5 *N* NaCl. By properly balancing the additions of 8% glycerol solution and 0.5 *N* NaCl solution, as shown in Table 2, the electrolyte concentration of the diluter was altered while the osmotic pressure was kept constant. To lower the

TABLE 2

Alteration of the electrolyte concentration of cysteine-treated nonfat dry milk solids diluter and its effect on the livability of bull spermatozoa

Treatment	Ml. added per 100 ml. standard diluter				pH	F.P.Δ ° C.	Mean livability[b]
	Water	0.5 *N* NaCl	8% Glycerol	Total			
1	4.0	12.0	0.0	16.0	6.38	−0.60	34.7
2	4.0	8.0	4.0	16.0	6.38	−0.60	37.5
3	4.0	4.0	8.0	16.0	6.38	−0.61	40.1
4	4.0	0.0	12.0	16.0	6.37	−0.61	39.0
5	0.0	−4.0[a]	16.0	16.0	6.36	−0.63	33.5

[a] Removed by dialysis. See text.

[b] Mean livability: Sum of mean motility readings of 10 ejaculates for day 1, 2, 3, 4, 6, 8, 10, and 12, divided by 8.

electrolyte concentration of the diluter, a 200-ml. aliquot of the diluter was dialyzed against 1,000 ml. of a 4.3% lactose solution. Exploratory trials indicated that 40 minutes dialysis at 20° C. resulted in a diluter which, by adding one further increment of glycerol, gave the same freezing point depression as the other treatments, indicating that electrolytes equivalent to one increment of 0.5 *N* NaCl had been removed by dialysis. Ten ml. of 0.1 *N* HCl was added to the lactose solution so that the more active hydrogen ions would not move into the lactose solution and alter the pH. Small amounts of water were added to the treatments that were not dialyzed, since the dialysis resulted in an increased volume. Data on the materials added and the mean livability results are presented in Table 2. There appeared to be a trend in the livability results indicating that the addition of 4.0 ml. of 0.5 *N* NaCl per 100 ml. of diluter was beneficial to spermatozoan livability, whereas smaller and larger amounts resulted in poorer livability.

The third of the three trials preliminary to the factorial experiment was performed at constant pH and electrolyte concentration but with variations in osmotic pressure. The osmotic pressure of the diluter was altered by the addition of a 12.5% glycerol solution to increase the freezing point depression and water to decrease the freezing point depression. A pH of 6.4 and an electrolyte concentration of 4.0 ml. of added 0.5 *N* NaCl per 100 ml. of diluter were used. The amount of added material was again kept constant at 16.0 ml. added per 100 ml. of diluter, with the exception of treatment No. 1, in which a larger amount of water was added to decrease the freezing point depression as much as possible. Osmotic pressure was varied from a freezing point depression of −0.43° C. to −0.77° C. Data concerning this trial are presented in Table 3. There were no apparent differences in livability due to differences in osmotic pressure within the ranges studied when osmotic pressure was varied by means of a 12.5% glycerol solution and by water.

With the preliminary experiments used as guides to the levels of pH, osmotic pressure, and electrolyte concentration, a factorial experiment was conducted in which three pH levels and three levels of electrolyte concentration were studied at two osmotic pressure levels. Alterations of the properties considered were brought about by the following means:

TABLE 3

Alteration of the osmotic pressure of cysteine-treated nonfat dry milk solids diluter and its effect on the livability of bull spermatozoa

Treatment	Ml. added per 100 ml. standard diluter				pH	F.P.Δ ° C.	Mean livability[a]
	Water	0.5 *N* NaCl	12.5% Glycerol	Total			
1	32.0	4.6	0.0	36.6	6.4	–0.43	39.1
2	12.0	4.0	0.0	16.0	6.4	–0.49	39.5
3	7.7	4.0	4.3	16.0	6.4	–0.59	39.8
4	4.0	4.0	8.0	16.0	6.4	–0.67	38.3
5	0.0	4.0	12.0	16.0	6.5	–0.77	40.8

[a] Sum of mean motility readings of 10 ejaculates for day 1, 2, 3, 4, 6, 8, 10, and 12 divided by 8.

1. When the diluter was first reconstituted, the pH, which averaged 6.4, was altered to the desired level by potentiometric titration with either 0.1 *N* HCl or 0.1 *N* NaOH. As in the preliminary trial, 0.1 *N* NaCl was used to equalize the amount of added electrolyte. The pH levels used were 6.2, 6.6, and 7.0.

2. The electrolyte concentration of the diluter was altered by the addition of 0.5 *N* NaCl. Levels used were 0.0, 5.0, and 10.0 ml. of 0.5 *N* NaCl added to 100 ml. of diluter.

3. Osmotic pressure was altered by the addition of 12.5% aqueous solution of glycerol by weight and the addition of water, while taking into consideration the effect on osmotic pressure of the electrolytes already added to satisfy the desired electrolyte concentration. Osmotic pressure levels used were approximately –0.50° C. and –0.70° C.

The total volume of material added was constant in all cases. In Table 4, information is presented on the desired level of pH, electrolyte concentration, and osmotic pressure; the amounts of added acid and base; the amount of added water and glycerol; and the actual levels attained. A summary of the livability results for each treatment over the 12-day storage period is presented in Table 5. The mean per cent motility tended to increase with some treatments during the first 2 or 3 days of storage; this observation is without meaningful explanation at this time.

Analysis of variance of the results showed no significant difference between pH levels or osmotic pressure levels, whereas the difference between levels of added electrolytes was significant ($P = < 0.05$). Least significant difference between the means showed both 5.0 and 10.0-ml. treatments to be significantly better than the 0.0-ml. treatment ($P = < 0.05$) with no significant difference between the 5.0 and 10.0-ml. treatments. There was a highly significant interaction between pH and electrolyte concentration. None of the interactions involving osmotic pressure were significant.

A comparison of pH and osmotic pressure measurements made at the beginning and end of the 12-day storage period indicated no appreciable change in either the pH or the osmotic pressure of the diluter during storage.

TABLE 4

Desired levels of pH, osmotic pressure, and electrolyte concentration for diluters in the 3×3×2 factorial experiment, amounts of various materials added to arrive at those levels, and the actual levels attained

Desired level of:			Ml. added per 100 ml. diluter:						Actual levels attained		
			pH adjustments			Os. pressure adj.					
pH	El. conc. ml. added 0.5*N* NaCl	O.P. F.P.Δ ° C.	0.1*N* HCl	0.1*N* NaOH	0.1*N* NaCl	e.c.a.[a] 0.5*N* NaCl	H_2O	12.5% Glyc.	pH	El. conc. ml. added 0.5*N* NaCl	O.P. F.P.Δ ° C.
6.2	0	−0.50	2.1	0	6.8	0	14	6	6.2	0	−0.48
6.2	0	−0.70	2.1	0	6.8	0	0	20	6.2	0	−0.71
6.2	5	−0.50	2.1	0	6.8	5	13	2	6.2	5	−0.47
6.2	5	−0.70	2.1	0	6.8	5	0	15	6.2	5	−0.67
6.2	10	−0.50	2.1	0	6.8	10	10	0	6.2	10	−0.50
6.2	10	−0.70	2.1	0	6.8	10	0	10	6.2	10	−0.64
6.6	0	−0.50	0	3.7	5.2	0	14	6	6.5	0	−0.47
6.6	0	−0.70	0	3.7	5.2	0	0	20	6.5	0	−0.69
6.6	5	−0.50	0	3.7	5.2	5	13	2	6.5	5	−0.46
6.6	5	−0.70	0	3.7	5.2	5	0	15	6.5	5	−0.66
6.6	10	−0.50	0	3.7	5.2	10	10	0	6.5	10	−0.53
6.6	10	−0.70	0	3.7	5.2	10	0	10	6.5	10	−0.65
7.0	0	−0.50	0	8.9	0	0	14	6	6.9	0	−0.47
7.0	0	−0.70	0	8.9	0	0	0	20	6.9	0	−0.70
7.0	5	−0.50	0	8.9	0	5	13	2	6.9	5	−0.45
7.0	5	−0.70	0	8.9	0	5	0	15	6.8	5	−0.67
7.0	10	−0.50	0	8.9	0	10	10	0	6.8	10	−0.51
7.0	10	−0.70	0	8.9	0	10	0	10	6.8	10	−0.65

[a] Electrolyte concentration adjustment.

TABLE 5

The effect of different levels of pH, electrolyte concentration, and osmotic pressure on the livability of bull spermatozoa (mean % motility of 12 bulls)

pH	0.5*N* NaCl	F.P.Δ	Days of storage at 4° C.								Mean livability
			1	2	3	4	6	8	10	12	
	(ml.)	*(−° C.)*									
6.2	0	0.50	52	50	48	48	30	16	15	8	33.4
6.2	0	0.70	51	50	50	47	28	17	14	9	33.3
6.2	5	0.50	56	52	52	50	32	22	15	9	36.0
6.2	5	0.70	58	56	57	50	31	22	17	11	37.8
6.2	10	0.50	54	60	58	54	36	19	12	11	38.0
6.2	10	0.70	54	56	55	52	52	17	13	9	36.0
6.6	0	0.50	51	51	53	46	36	22	18	14	36.4
6.6	0	0.70	51	52	49	48	38	25	20	13	37.0
6.6	5	0.50	55	58	58	54	42	24	17	14	40.2
6.6	5	0.70	58	60	54	51	38	26	24	13	40.5
6.6	10	0.50	58	60	56	54	38	26	15	8	39.4
6.6	10	0.70	59	58	56	53	41	24	17	12	40.0
7.0	0	0.50	52	45	42	44	28	18	16	9	31.8
7.0	0	0.70	48	45	46	42	29	19	15	11	31.9
7.0	5	0.50	53	58	58	53	38	26	18	12	39.5
7.0	5	0.70	54	57	57	52	38	28	18	13	39.6
7.0	10	0.50	58	57	58	51	42	25	19	9	39.9
7.0	10	0.70	60	59	57	50	38	25	18	15	40.2

DISCUSSION

The most interesting observation from these trials is that bull spermatozoa live equally well at varying levels of osmotic pressure but are obviously sensitive to varying levels of electrolyte concentration. This is evidence that the materials used to bring about the osmotic pressure are considerably more important than the level of osmotic pressure attained. The fact that osmotic pressure is not significant in these data and does not interact significantly with any other property of the diluter indicates that within the ranges studied, osmotic pressure, of itself and independent of electrolyte concentration, is of no consequence to spermatozoan livability.

Also of interest is the highly significant interaction between pH and electrolyte concentration. The addition of electrolytes is considerably more important at a pH level of 7.0 than at 6.2 or 6.6. It seems, then, that higher pH levels require higher levels of electrolytes, or vice versa.

That the pH of the milk diluter did not change during storage is contradictory to the speculation of some workers who have indicated that changes in the pH of milk diluters during storage might become a limiting factor in spermatozoan livability. It would seem that no improvement could be expected in a milk diluter by increasing the buffering capacity of the diluter. Although the change in the osmotic pressure of a diluter during storage would apparently be a poor quality test of a diluter, since osmotic pressure by itself has no apparent role in spermatozoan livability, still it is interesting to note that the osmotic pressure of the diluter used in the factorial experiment changed less during a 12-day storage period than the compounded egg yolk–buffer diluters investigated by Smith *et al. (6)* during a 10-day storage period.

Since the pH of the unaltered cysteine-treated nonfat dry milk solids diluter is approximately 6.4, and the osmotic pressure approximately –0.50° C. in terms of freezing point depression, no improvement could be expected in the ability of this diluter to support livability by alterations in either of these factors or, as previously mentioned, by alteration of the buffering capacity of the diluter. Increasing the electrolyte concentration of the diluter by the addition of 0.5 *N* NaCl, however, would result in slightly improved livability.

SUMMARY

The effects of alterations in the pH, osmotic pressure, and electrolyte concentration of a cysteine-treated nonfat dry milk solids diluter on the livability of bull spermatozoa have been investigated. There were no differences in livability between the two osmotic pressure levels studied (approximately –0.50° C. and –0.70° C.) and no appreciable differences among the pH levels studied (6.2, 6.6, and 7.0). The addition of either 5.0 or 10.0 ml. of 0.5 *N* NaCl solution to 100 ml. of diluter resulted in significantly improved livability. A highly significant interaction between pH and electrolyte concentration indicated that higher pH levels require higher levels of electrolytes. Neither the pH nor the osmotic pressure of the diluter changed appreciably during a 12-day storage period. It was concluded

that no improvement in spermatozoan livability can be expected by alteration of the pH, osmotic pressure, or buffering capacity of the diluter employed but that the addition of 0.5 *N* NaCl results in slightly improved livability.

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EFFECTS OF SUPPLEMENTING A CALF RATION WITH TRACE MINERALS, AUREOMYCIN,[1] AND OTHER DIETARY CONSTITUENTS AS MEASURED BY GROWTH AND FEED CONSUMPTION[2]

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Because of the interest in aureomycin for feeding dairy calves *(13)*, it seemed advisable to determine what effect this antibiotic would have on hematopoiesis and microscopic anatomy of calves. In a preliminary experiment the blood characteristics of two calves (3 months old) were studied prior to aureomycin feeding. It was found that the values obtained for several of the blood characteristics were below those commonly reported for calves. Feeding trace minerals to these calves produced hematological changes which appeared beneficial. Although there have been a few reports *(14)* on the effect of the addition of trace mineral elements to calf rations known to be deficient in these minerals, there is a paucity of information on the effect of fortifying common calf rations with trace minerals. This experiment was initiated to study the effects of supplementary trace minerals only and a combination of trace minerals together with major minerals, vitamins, and aureomycin upon certain physiological responses of the calf. Data pertaining to the growth and feed consumption of the calves are presented in this paper. Those related to the hematology, histology, chemical composition of the meat, and weight of certain body organs will be reported later.

EXPERIMENTAL PROCEDURE

Twenty-seven Holstein and Ayrshire calves were used. Three test rations were assigned at random within breed-sex groups. Each group contained two Holstein females, two Holstein males, three Ayrshire females, and two Ayrshire males. Calves in Group I (basal) were removed from their dams at birth, placed in individual pens, and fed their mothers' colostrum for the first 3 days at the daily rate of 1 lb. per 10 lb. body weight. Beginning with the fourth day, they were fed whole milk at approximately 10% of body weight daily for the next 4 weeks. After the fourth week the rate of milk feeding was gradually reduced until a total of 350 lb. had been consumed. Milk feeding ceased between 6 and 8 weeks of age. Good quality alfalfa hay and a calf starter were fed ad libitum,

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beginning at 1 week of age. The starter was calculated *(9)* to contain 22% crude protein and consisted of 195 parts by weight of cracked yellow corn, 200 of oats, 150 of wheat bran, 50 of dried skimmilk, 240 of soybean oil meal, 50 of molasses, 70 of dehydrated alfalfa chops, 30 of brewers' yeast, 5 of limestone, 5 of steamed bone meal, 5 of salt, and 1.25 of irradiated yeast. Beginning at the 19th week of age, the calves were changed from the starter to a grower ration consisting of 200 parts by weight of corn, 200 of oats, 200 of wheat bran, 175 of soybean oil meal, 6 of salt, and 6 of steamed bone meal. This ration was calculated *(9)* to contain 18% crude protein. Intakes of starter and grower rations were limited to a maximum of 5 lb. per calf per day. Calves in Group II received, in addition to the above basal ration, iron, copper, cobalt, iodine, manganese, and zinc (trace minerals). Calves in Group III received the basal ration plus these trace minerals, and in addition major minerals (calcium, phosphorus, magnesium chloride), vitamins (A, B-complex, C, D, E, K), and aureomycin.

The nutritive value of the basal ration was considered to be approximately optimal, based upon present National Research Council recommended nutrient allowances *(10)*. Therefore, any additions, as indicated for Groups II and III, were arbitrarily selected and presumed to be in excess of the accepted daily requirements. The supplementation of the ration for calves in Group III with major minerals, aureomycin, and vitamins, in addition to trace minerals, was made in an attempt to prevent any possible deficiency of the basal ration. The number of blood and tissue analyses required obviated the feasibility of dividing this group according to the respective types of supplements.

The trace minerals were at first administered daily by capsule from birth. When the starter consumption was great enough to provide a carrier, the supplement was furnished in the starter and capsule feeding was discontinued. The quantities of trace minerals in elemental form supplied daily in milligrams per 100 lb. body weight were 200 of iron, 20 of copper, 20 of manganese, 10 of cobalt, 10 of zinc, and 0.19 of iodine. Iron, copper, manganese, and zinc were supplied as sulfates, cobalt as a chloride, and iodine as potassium iodide in stabilized iodized salt. These quantities supplied approximately 2.5 times the amount of iron, copper, and manganese as fed by Herman *(5)* to prevent anemia in calves on milk alone, and twice the amount of iron that Thomas *et al. (16)* found would increase the hemoglobin values of somewhat anemic calves. The quantity of supplementary cobalt was similar to that fed by Duncan *et al.. (3)* to alleviate symptoms of cobalt deficiency in dairy cattle.

The major minerals were administered in a manner similar to that employed with the trace minerals. The quantities of major minerals in elemental form supplied daily in grams per 100 lb. body weight were 3 of calcium, 2.32 of phosphorus, 0.3 of magnesium, and 2.5 of sodium chloride. Calcium and phosphorus were supplied as dicalcium phosphate, sodium and chlorine as sodium chloride, and magnesium as heavy magnesium oxide. The above amounts of calcium, phosphorus, and magnesium including that present in the basal ration slightly exceeded the National Research Council's recommended calcium and phosphorus allowances *(10)* and the magnesium requirement suggested by Huffman *et al. (7)*.

TABLE 1
Supplemental vitamins fed daily to calves in Group III

Vitamin	Quantity/100 lb. body wt.	Vitamin	Quantity/100 lb. body wt.
	(mg.)		*(mg.)*
Choline	1300.0	Pyridoxine	7.0
Inositol	130.0	α-tocopherol	5.0
Ascorbic acid	65.0	Thiamine	4.0
Ca-pantothenate	40.0	2-methyl-1, 4-napthoquinone	1.3
Nicotinic acid	15.0	Folic acid	0.3
Para-amino benzoic acid	13.0	Vitamin B_{12}	0.1
Riboflavin	7.0	Biotin	0.05
	Vitamin A 10,000 USP units		
	Vitamin D 400 USP units		

The kind and amount of vitamins fed to the calves in Group III (Table 1) were based on the work of Wiese *et al. (17)* and Johnson *et al. (8)*, in which it was demonstrated that these amounts were optimal for calves on synthetic diets. Therefore, these amounts were adequate in themselves and were in addition to those contained in the basal ration. Vitamins A, E, and K (in liquid form) were given daily by capsule throughout the experiment. The other vitamins were in the form of a powder and were given daily by capsule until each animal had begun to consume the entire quantity of starter, at which time the vitamin mix was placed in the starter or grower ration.

Fifty mg. of crystalline aureomycin HCl was fed daily to calves in Group III in a manner similar to that used for administering the trace mineral mix.

The calves were weighed at weekly intervals. Records were kept of all feed consumed. The duration of the experiment was from birth to 24 weeks of age.

RESULTS AND DISCUSSION

The calves involved varied in size because of differences in breed, sex, and birth weight. Therefore, all weight data were converted to a comparable basis by converting the weekly body weights of each calf into a percentage of original birth weight, the original birth weight being considered 100 for each calf, regardless of size.

Graphical analysis of the average weights of the three groups of calves (Figure 1) indicates a tendency for the growth increase to be linear during the first 8 weeks and during the last 16 weeks, separately. This interpretation is supported by regression analysis for each group of calves during each of the two periods.

During the first 7 weeks after birth the calves in Groups II and III grew at a significantly faster rate than those in Group I (Table 2), but it was not until the eighth week that the spread in average size among the groups was statistically significant. The cessation of milk feeding at about the eighth week seemed to occur at about the time that the calves fed trace minerals (Groups II and III) showed highly significant differences in growth rate compared with calves not supplemented (Group I). The significance of these two occurrences can not be

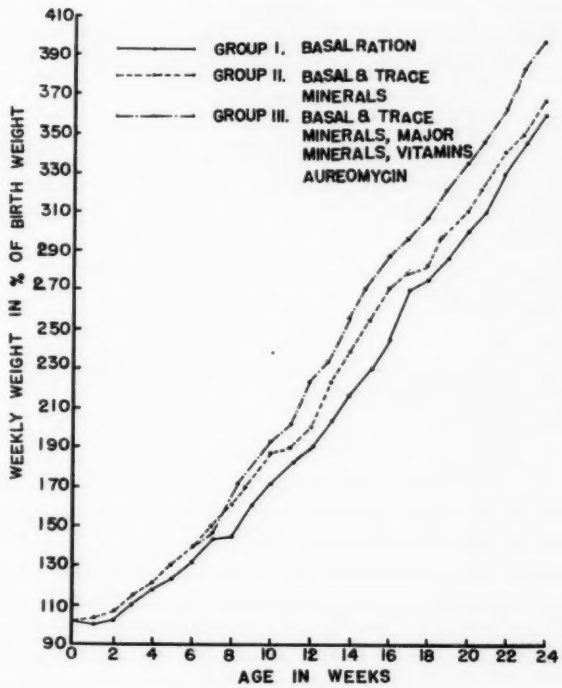

FIG. 1. Comparative growth responses of three groups of calves receiving a basal ration (Group I), the basal plus trace minerals (Group II), or the basal plus trace minerals, major minerals, vitamins, and aureomycin (Group III).

determined from the data available. After 8 weeks, calves in Group II continued to average larger than those in Group I (basal), but comparatively there was a gradually diminishing growth rate of calves in Group II (Table 2). At 24 weeks there was practically no difference in the size of the calves in these two groups. Calves in Group III continued to grow at a significantly faster rate than those in either of the two other groups and at 24 weeks were decidedly larger than the calves in either Group I or II.

The improvement in growth for calves in Groups II and III during the early weeks of life is supported by blood data (to be reported later) which show that the feeding of trace minerals to calves (Group II) and the feeding of trace minerals plus major minerals, vitamins, and aureomycin (Group III) prevented, to a large extent, the drop of hemoglobin and red blood cell count that usually occurs during the first few weeks of a calf's life *(16, 18)*. Of course, the trace minerals added were present in varying amounts in both the roughage and grain, and to what extent additional trace minerals would be justified will depend on further research. The fact that the rate of growth of calves in Group II (trace

TABLE 2

Regression analyses of the effect of supplementing a basal calf ration with trace minerals alone and in combination with major minerals, vitamins, and aureomycin on the growth rate of calves from birth to 24 weeks of age

Slope of growth line					
Birth to 8 wk.				9 to 24 wk.	
Group	Treatment	No. of calves	Regression Coefficient *b*[a]	Group	Regression Coefficient *b*
II	Basal ration plus trace minerals	9	9.06 < ns	III	14.41 < **
III	Basal ration plus trace minerals, major minerals, vitamins and aureomycin	9	8.99 < **	I	13.53 < **
I	Basal ration	9[b]	6.80	II	12.67

[a] Analysis of growth data employing the regression of weekly wt/lb of birth wt. on age.
[b] One calf died of an impacted abomasum.
** = Difference significant at the 0.01 level.
ns = Difference not significant.

minerals) tended to decrease gradually after 8 weeks compared with the calves on the basal ration raised the question as to the appropriate amount and length of time that additional minerals should be fed.

Further fortification of the basal ration by the addition not only of the trace minerals but also of major minerals, vitamins, and aureomycin resulted in further improvement in rate of growth and greater average size for calves in this group than for either of the two other groups. It was not possible to determine whether this improved growth of calves in Group III should be ascribed to trace minerals plus any one of the other supplements or a combination of several. The fact that in previous experiments at this station *(1, 2)* accelerated growth of similar magnitude has been obtained by the addition of aureomycin alone would tend to cast some doubt on the beneficial effects of some of the supplements used in the ration for Group III, other than trace minerals. Also, several investigators *(4, 6, 11, 12, 15)* have found that supplementing calves with vitamins does not increase growth above that obtained when calves are fed colostrum and standard rations. Additional experiments in which the influence of the other supplements are measured separately seem advisable.

The apparent thrift of the calves in Groups II and III would suggest no detrimental effects resulting from the supplements added to the basal ration within the limits of this feeding period.

It was assumed that some of the benefits derived from feeding a combination of vitamins, trace minerals, major minerals, and aureomycin might well be reflected by feed consumption and feed efficiency of each animal. The average weekly intake of hay and grain (starter or grower) by the calves in each of the three groups is shown in Table 3. Since whole milk was fed on a body weight basis, the milk intake was not studied. The calves in Groups I and III consumed similar amounts of hay and grain. Calves in Group II receiving trace-minerals consumed less hay but more grain than the calves in the two other groups. An

TABLE 3

Effect of supplementing a basal calf ration with trace minerals alone and in combination with major minerals, vitamins, and aureomycin on feed intake and efficiency of gain of calves from 1 to 24 weeks of age[a]

Group	Treatment	No. of calves	Av. weekly intake/calf /100 lb. body weight		TDN /lb. gain	Dig. protein /lb. gain
			Hay	Grain		
			(lb.)	*(lb.)*	*(lb.)*	*(lb.)*
I	Basal ration	9[b]	9.3	14.5	2.95	0.68
II	Basal ration plus trace minerals	9	8.8	15.3	3.02	0.69
III	Basal ration plus trace minerals, major minerals, vitamins, and aureomycin	9	9.3	14.3	2.79	0.64

[a] None of these differences was statistically significant.
[b] One calf died of an impacted abomasum.

analysis of variance of the data showed that none of these differences was statistically significant.

The calves receiving the trace mineral supplement (Group II) required slightly more total digestible nutrients and digestible protein per pound of gain than the Group I control calves (Table 3) whereas the calves receiving the trace and major minerals, aureomycin, and vitamin supplement (Group III) made the most efficient gains. However, an analysis of variance of these data showed that these differences were not significant.

SUMMARY

Twenty-seven dairy calves were divided into three ration groups. Calves in Group I received a standard basal ration. Calves in Group II received the basal ration plus trace minerals (iron, copper, cobalt, iodine, manganese, and zinc). Calves in Group III received the basal ration plus these trace minerals, and, in addition, major minerals (calcium, phosphorus, magnesium, and sodium chloride), vitamins (A, B-complex, C, D, E, K), and aureomycin. The duration of the experiment was from birth to 24 weeks of age.

During the first 7 weeks after birth the calves in Groups II and III grew at a significantly faster rate than those in Group I (basal), but it was not until the eighth week that the spread in average size among the groups was statistically significant. After 8 weeks, calves in Group II continued to average larger than those in Group I (basal) but comparatively there was a gradually diminishing growth rate of calves in Group II. At 24 weeks there was practically no difference in the size of the calves in these two groups. Calves in Group III continued to grow at a significantly faster rate than those in either of the two other groups and at 24 weeks were decidedly larger than the calves in Groups I and II.

Thus the addition of selected trace minerals to the basal ration resulted in improved size, but there was a comparative trend to slow up in growth rate by calves in Group II after 8 weeks of life.

Further fortification of the basal ration by the addition not only of the trace minerals but also major minerals, vitamins, and aureomycin resulted in further improvement in rate of growth and greater average size for calves in this group than for either of the two other groups.

Small differences resulted among the groups in feed consumption and efficiency of gain. However, none of these differences was statistically significant.

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EFFECTS OF FEEDING GRASS AND OF SANITATION ON GROWTH OF YOUNG DAIRY CALVES UNDER TWO SYSTEMS OF MANAGEMENT

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Recent studies have shown several advantages of open type housing (open sheds and portable outside pens) over the conventional type closed housing for raising dairy calves. Some apparent advantages of the open type housing for raising calves include diminution in respiratory diseases *(5, 12)*, in parasites *(5)*, and in mortality rates *(5, 8, 12)*. In addition, calves raised in portable outside pens grew more rapidly than calves raised in conventional housing *(5)*. The differences in growth rates possibly were affected by coccidial infections, which were greater for the calves housed in a conventional barn. Nevertheless, under the conditions of the experiment *(5)*, no evaluations could be made of the effect on growth of the fresh supply of grass that was available weekly to the calves raised in portable outside pens. A survey of the literature failed to elucidate the problem but did reveal that fresh green forage *(1)* and forage juices *(10, 13)* increased the rate of growth of monogastric animals over that of similar animals on basal diets.

The investigation reported herein was initiated to study the effect of grass on the growth rate of calves raised in conventional housing and of calves raised in portable outside pens. Subsequently, general sanitation was included as an additional treatment variable.

EXPERIMENTAL PROCEDURE

The study extended over a 2-year period and involved 36 calves that were less than 2 weeks of age at the beginning of the experimental treatments. During each year there were three replications per treatment, two of Jerseys and one of crossbred dairy calves. The crossbred calves used during the first year were Holstein × Jersey, and those used during the second year were Brown Swiss × Jersey. Each year, within replications, the calves were assigned at random to the treatment variables.

The variables studied during both years included housing, grass as a dietary supplement, and methods of feeding grass, i. e., clipped or grazed. The treatments are defined as follows:

(a) Inside, or conventional type, housing.

I, fed basal diet only.

IG, fed basal diet supplemented with 1 lb. of grass cut and fed fresh daily.

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(b) Open, or portable pen type, housing located on an isolated area that was plowed and seeded to Italian ryegrass and crimson clover about 6 weeks prior to use in the study.

OS, fed basal diet only; locations for portable pens scraped free of vegetation before placing calves on area.

OSG, fed basal diet supplemented with 1 lb. of grass cut and fed fresh daily; area treatment similar to that of OS.

OGr, fed basal diet, and grass in pen area was left for grazing.

(c) Open, or portable pen type, housing located on an area covered with Bermuda grass stubble and frequented by mature cattle, i. e., a contaminated area.

OC, fed basal diet only.

OCG, fed basal diet supplemented with 1 lb. of grass cut and fed fresh daily.

Thus, the five treatment groups for the 2-year period were: I, IG, OS, OSG, and OGr. The additional treatment groups during the second year were: OC and OCG.

The land area for the portable pens, except those in treatments OC and OCG, and the barn and exercise lot used for the conventionally housed calves have been described elsewhere *(5)*. The portable pens housing the experimental calves were shifted at weekly intervals as recommended *(3, 6)*. The lot used for exercising conventionally housed calves was scraped free of vegetation at weekly intervals. The Bermuda grass stubble on the areas occupied by pens housing calves in the OC and OCG treatment groups was approximately ½ inch high and did not provide an appreciable amount of nutrients for the calves. Calves assigned to treatments involving portable outside pens remained in the pens 24 hours each day, whereas calves assigned to treatments involving conventional housing were turned outside to exercise in the scraped lot daily except during inclement weather.

The basal diet was the same for calves assigned to all treatments during each year; however, the basal diet varied for the 2 years. Each calf received colostrum from its dam and subsequently was fed liquid milk (whole or reconstituted). Liquid milk feeding was discontinued at 4 months of age during the first year. Since only whole milk was fed during the second year, the liquid milk feeding period was reduced to 7 weeks. Alfalfa hay and a concentrate containing approximately 16% crude protein were introduced into the diet when the calves were 2 weeks of age. The quantity of concentrate fed was limited to 4 lb. daily, but the consumption of hay was limited only by the appetite of the calf. During the first year, with the exception of a 3-day interval during the period of liquid milk feeding, water was offered to the calves twice daily in individual buckets. Water was offered to the calves twice daily throughout the second year.

The grass fed to calves on treatments IG, OSG, and OCG was clipped from areas that were to be scraped. The green forage that was clipped and fed fresh

5 days per week during the first year and daily during the second year was introduced into the diet when the calves were 35 days of age.

The quantity of all dietary ingredients fed and refused was recorded, and the quantity of grass grazed by calves on treatment OGr was estimated. Total digestible nutrient intakes of the calves were calculated from Morrison's digestibility coefficients *(11)*.

The number of oocysts of coccidia and the number of worm eggs in feces from the calves were determined as described previously *(5)*; however, the frequency of sampling for oocysts of coccidia was reduced to once weekly. Worm egg counts were determined only once during the study, at which time the range in ages of the calves was 82 to 160 days.

All calves were weighed at the time they entered the study and at regular intervals until they were removed from the experiment at 180 days of age. Other body measurements including height at the withers, heart girth, and barrel girth were taken at regular intervals during the second year only.

RESULTS AND DISCUSSION

First year. The relationship between treatment variables and weight gains of the calves is shown in Table 1. Average gains in body weight of calves on treatment IG were from 37.6 to 51.0 lb. greater than the average gains of calves on other treatments, but the differences were nonsignificant.

TABLE 1

Relationship between treatments and weight gains of dairy calves during the age range of 14 to 180 days

Treatment	Mean gain in body weight
	(lb.)
I	152.6
IG	197.3
OS	159.7
OSG	157.7
OGr	146.3
L.S.D., P = 0.05	58.7

The absence of a detectable effect of type of housing on weight gains in this study is at variance with a previous report *(5)*. There was a difference in the procedures for the two studies that possibly accounts for the variations in responses. In the earlier study, the lot used for exercise of calves raised in conventional housing had a few sprigs of vegetation. Frequently, the calves were observed nibbling at these sprigs. Possibly the vegetation, which was not an important source of energy, provided a source for parasitic infection and directly or indirectly depressed the rate of growth of conventionally housed calves. Also, in the earlier study, the calves were assigned to the experiment at a younger age than calves in this study.

The possibility of vegetation stubble affording a means of infection of the calves with parasites and an accompanying depression of growth rate suggested

the need for information on the effect of general sanitation on growth response of calves housed in portable outside pens. Treatments OC and OCG, which have been described previously, were included in the second year's study to evaluate this problem.

Second year. Lethargy and anorexia were common symptoms of Jersey calves assigned to treatments OC and OCG. Sulfaguanadine was administered to two of these calves, one on each treatment, that developed severe cases of scours. When some of the calves on treatments OC and OCG were approximately 120 days of age extreme weakness was apparent and it was necessary to remove them from the experiment to prevent death. Consequently, data for the second year's work are presented for two age ranges, i. e., 14 to 120 days, which includes all treatments, and 14 to 180 days, which includes only the treatments studied during the first year.

During the age range 14 to 120 days, calves assigned to the OCG treatment gained significantly less weight than calves on treatments OS and OSG. The average body weights of calves assigned to treatments I, IG, and OGr were 19 to 33 lb. greater than the average weights of calves on treatments OS and OSG, but the differences were not significant statistically. Subsequently, in order to obtain a more sensitive test of dietary, housing, and management effects, data were rearranged to take advantage of the factorial aspects of the design. The results of these analyses are presented in Table 2. The average gains in body weight of calves raised in open housing, on an area that was isolated from older cattle, were slightly greater than those of calves raised in conventional housing. Also, average weight gains of calves on diets that included grass were slightly greater than those of calves that did not receive grass in their diet, except for calves raised in portable outside pens on an isolated area. The differences in weight gains associated with diet and with type of housing, however, were not significant. On the other hand, the analysis of data in the factorial arrangement shows that management (sanitation) practices had a significant effect on rates of gain in body weight. Calves raised in portable outside pens on an isolated area grew significantly more rapidly than the calves raised in portable pens located on an area accessible to older cattle.

TABLE 2

Relationship between diets, housing, and sanitation and mean gains in body weight of calves from 14 to 120 days of age

Diet	Inside housing	Open housing[a]		Mean[b]
		Clean area	Contaminated area	
	(lb.)	*(lb.)*	*(lb.)*	*(lb.)*
No grass	108.3	118.0	88.0	104.8
Cut grass[c]	108.0	118.0	75.3	100.4
Mean[d]	108.2	118.0	81.6	102.6

[a] See procedure for description of areas.

[b] L.S.D., P = 0.05 for comparing diet means is 27.2.

[c] Body weight gains for treatment OGr is 107.0 lb. and L.S.D., P = 0.05 for comparing individual treatment means is 38.5.

[d] L.S.D., P = 0.05 for comparing housing means and for comparing management means is 22.1.

During the age range 14 to 120 days, gains in height at the withers of calves assigned to treatments I, IG, OS, and OSG were significantly greater than those of calves assigned to treatments OC and OCG. Also, gains in height at the withers by calves on the OGr treatment were significantly greater than those of calves on the OC treatment but did not differ significantly from that of calves on other treatments.

Dietary, housing, and sanitation treatments did not affect significantly the gains in heart girth or the gains in barrel girth during the age range 14 to 120 days. This relationship persisted during the age range 14 to 180 days (Table 3).

TABLE 3

Relationship between treatments and growth response of dairy calves from 14 to 180 days of age

Gains in body measurements	Treatments					L.S.D. P = 0.05
	I	IG	OS	OSG	OGr	
Weight *(lb.)*	189.7	198.3	181.3	176.0	186.7	43.20
Height at withers *(in.)*	9.9	8.9	7.5	9.2	8.7	1.27
Heart girth *(in.)*	15.3	15.7	14.7	15.5	15.8	3.20
Barrel girth *(in.)*	23.2	23.5	23.5	24.0	24.2	5.31

As in the first year's findings, differences in weight gains associated with treatments I, IG, OS, OSG, and OGr were not significant (Table 3). In the second year, calves assigned to treatment OS made less gain in height than calves assigned to other treatments, except OGr. This difference in gain in height at withers was associated with the open housing but was not consistent with other body measurements.

Coccidia oocysts were found more frequently and in greater numbers in feces from calves raised in portable outside pens located on contaminated soil than in feces from calves assigned to other treatments. In a number of the fecal samples from the calves assigned to the contaminated area, however, coccidia oocysts were not detected. In general, oocyst counts were low in feces from calves assigned to the other treatments.

Also, the worm egg count in feces from the calves was either negative or very low. Calves on treatments OC and OCG were in a weakened condition and showed symptoms suggestive of parasitism at the time the fecal samples were collected.

Two year summary. During the second year, calves assigned to all treatments made greater gains in weight than calves assigned to the same treatments during the first year. The 2-year weight gains (unadjusted, Table 4) indicate that the weight increases by conventionally housed calves that received grass (treatment IG) were significantly greater than those of calves in portable outside pen treatments. Differences in weight gains between treatments I and IG were nonsignificant. The 2-year data clarified a trend that was suggested by growth data for the individual years and emphasize the necessity for caution in drawing conclusions from data involving only a small number of animals. A perusal of the feed intake and weight gain data suggested that some of the variation in weight gains

of the calves between years and between treatment groups was associated with differences in feed intake. In order to take this variation into account, the nutrient intake and gains in body weight data were subjected to covariance analyses *(14)*. The adjusted mean gains in body weight for five treatments during the 2-year period (Table 4) reveal that calves housed inside gained significantly more weight per pound of digestible nutrients consumed than calves raised in open housing that received grass, cut or grazed. On the other hand, gains in body weight by calves raised in open housing without grass (treatment OS) were intermediate and did not differ significantly from those of calves on other treatments.

TABLE 4

Relationship between TDN intake and mean gains in body weight by calves during the age range 14 to 180 days

Treatment	Unadjusted gain in body weight	Intake of TDN	Adjusted gain in body weight
	(lb.)	*(lb.)*	*(lb.)*
I	171.5	448.7	194.3
IG	195.7	503.5	196.0
OS	169.8	504.2	169.8
OSG	167.2	544.5	150.6
OGr	165.2	520.0	158.7
L.S.D., P = 0.05	27.2	——	28.7

The adjusted weight gain data indicated that the additional protection provided by conventional housing over that provided by open housing (portable outside pens) was beneficial to the calves, at least from the standpoint of weight gains. These findings are similar to those reported by Erb *et al. (8)*, in which Holstein calves housed in open sheds required more TDN per pound of gain than calves raised in closed barns. Erb *et al. (9)* stated that undoubtedly some of the extra feed consumed by calves in open housing was due to energy used through exercise. However, this relationship did not exist between the inside housing (treatments I and IG) and open housing (treatments OS, OSG, and OGr), since the calves in treatments with the most efficient utilization of TDN for gains in weight were the only calves that had access to a large exercise lot daily. Also, the 2-year unadjusted weight gain data, reported herein, which showed no apparent advantage of portable pens over conventional housing, are similar to the findings reported by Murley *et al. (12)* but are at variance with previous findings of Davis *et al. (5)*. Murley *et al.* compared open and closed barns, whereas Davis *et al.* compared portable outside pens and a conventional type calf barn. In the latter study, the effects of housing, parasitic infection, and dietary differences were confounded, whereas in the present study and in the study reported by Murley *et al.* the effects of housing appear to be separated from other variables. Thus, it is indicated that the type of housing in itself does not affect growth response of calves but that conditions associated with a given type of housing may have an important bearing on growth. The results reported herein indicated that some of the problems associated with a barn housing situation *(5)* may be overcome by careful management.

Apparently grass did not supply the calves with required nutrients, other than those supplied by the basal diet, but did promote a greater feed intake by the calves. This negative growth response to grass feeding agrees with recent reports in which there was no apparent advantage to providing grass juice concentrate *(7)* or pasture *(12)* for young calves. Conversely, the growth response of calves to grass feeding in this study is in contrast to the growth impetus of grass juice concentrate for chicks *(10)* and poults *(13)* and of grass for pigs *(1)*. This lack of a growth stimulus from the addition of grass to the diet of calves in this study emphasizes further the basic differences in nutrient requirements that must be supplied through feeds to young ruminants and to young animals with simple stomachs. The greater weight gains of calves raised in portable outside pens than those raised in conventional housing in an earlier study *(5)* possibly were the result of an indirect effect of grass intake on consumption of other feeds by calves housed in portable pens.

The phase of this study that involved comparisons of a clean, isolated area and a contaminated (accessible to older cattle) area for locations of portable outside pens showed that general sanitation is an important consideration in raising healthy, vigorous calves. Routine fecal examinations for oocysts of coccidia and a spot check for worm eggs when calves on treatments OC and OCG were in a weakened condition indicated that these parasites did not have a significant bearing on the growth response of the calves. According to Davis *(4)*, however, possible involvement of internal parasites cannot be eliminated because of the interval between examinations for oocysts of coccidia and the single check for worm eggs. Nevertheless, it appears probable that the weakened condition of calves raised in portable outside pens located on the contaminated area was caused by a bacterial or virus infection. The growth data indicate that calves cannot be raised successfully in portable outside pens that are located on areas frequented by older cattle. A growth-depressing infection in chicks housed in habitually used poultry premises may be counteracted by penicillin *(2)*. This suggests that the deleterious effect of the contaminated area on growth and general health of calves possibly could be offset by feeding antibiotics. However, an isolated area is recommended *(3)* for location of portable outside pens.

SUMMARY

Growth response of calves raised in a conventional calf barn and those raised in portable outside pens located on clean soil was similar. Grass fed at the rate of 1 lb. per calf daily increased the TDN intake but did not affect feed utilization or rate of gains in body weight within housing groups. Raising calves in portable outside pens located on an area frequented by older cattle depressed gains in weight and gains in height at the withers when compared with raising calves in portable outside pens located on a clean, isolated area. Gains in body weight per pound of TDN consumed were greater for calves raised in conventional housing than for calves raised in portable outside pens.

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PROTEIN PRODUCTION IN THE BOVINE. COMPARISON OF DAILY PROTEIN, FAT, AND MILK PRODUCTION DURING THE ENTIRE LACTATION PERIOD[1]

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Because of economic considerations, the butterfat producing ability of the dairy cow and the factors influencing it have been extensively studied. Other components of milk have not received this attention, and, consequently, much less is known about their production during the lactation cycle. Although much indicatory work has been done concerning the protein of milk *(1-6, 10-21)*, most studies have been concerned primarily with the composition and not with the total amount of protein produced by a given animal. As a result, many years of selective breeding for optimal fat production have been carried out with little or no consideration given for protein production. With the recent changes in the economic importance of butterfat and the realization that in the future protein may become more of a limiting factor in the world's food supply, the ability of the dairy cow as a synthesizer of high grade protein should receive more attention.

This investigation has been undertaken with the object of studying protein production in the bovine, and in this manuscript preliminary background experiments are reported which compare the protein production of the dairy cow with the total milk and fat produced during the lactation period. Rather than study a large number of cows less intensively, it was deemed advisable to concentrate on a few and by frequent analyses establish precisely their protein production during the complete lactation period. Studies on the total protein production of a larger number of animals will be reported in subsequent reports.

METHODS AND PROCEDURES

Four healthy cows of the University herd were chosen which could be studied through an entire lactation period and into their next lactation. Two of the animals were heifers carrying their first calves and two were cows which had been dried off approximately 2 months previous to their expected parturition date. Cow A aborted after 267 days in the lactation period from unknown causes; consequently, her lactation period was extended to 377 days, when she was dried off and removed from this experiment. Cows B and D were milked through the normal dry period and their production studies were carried on into the next lactation. Cow C was dried off about 2 months prior to her expected parturition date, and her production studies were also continued at the start of her next lactation. Pertinent facts concerning these cows prior to and during their first lactation under study are given in Table 1. The total production data for each

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cow was calculated on the basis of a lactation period, including the first through the 345th day.

The cows were carefully watched at parturition, and the calves were immediately confined. The calves did not receive any of the colostrum or milk until it had been removed from the dam and weighed and a sample placed in the refrigerator for the laboratory analyses. Samples were taken from each milking for the first week and then at less frequent intervals during the entire lactation period. The morning and evening milk for each day was warmed and composited into one sample according to weight before analyzing. For Cow B, which calved late in the day, the yield on the day of calving (0 day) was considered to be this evening's milking and the next morning's milking. Fat was determined by the Babcock procedure. Total protein, casein, serum (whey) protein, and nonprotein nitrogen were evaluated by the procedures of Rowland *(17)* with a semimicro Kjeldahl digestion and distillation apparatus *(7)*. Unless specified otherwise, total protein includes the casein, serum protein, and nonprotein nitrogen fractions, and all were calculated as protein (N × 6.38).

RESULTS AND DISCUSSION

A comparison of the production curves in the left-hand portion of Figures 1a, 2a, 3a, and 4a for the four cows in their first lactation shows that they were essentially similar in their major characteristics. The total pounds of milk and grams of fat produced per day by each cow demonstrated the characteristic increase during the first month or so after parturition. With the exception of Cow A, which had earlier aborted and hence was not nearing the end of a normal lactation, the amount of fat produced per pound of milk also showed an increase near the end of the lactation period. Thus, considering the amount of milk and fat produced per day and their relationship during the lactation period, these cows appeared to be normal.

The amount of total protein produced per day during the first month or so, however, presents a different picture than the amount of milk and fat produced during this time. Even though considerable variation was observed the first few days, the daily output of total protein was essentially at its maximum level on the day of parturition and then declined during the lactation period. The curves in Figures 1b, 2b, 3b, and 4b show, however, that during the first few days the kind of protein present in the milk varied tremendously. The production of casein increased for the first few days as did the output of the nonprotein nitrogen, but the simultaneous decrease in the serum proteins maintained the total protein level rather constant or caused it to decrease.

The production curves for these cows carried into the following lactation are shown in the right-hand portion of Figures 2, 3, and 4. The production curves for Cow B reflect the influence of milking through what normally would have been the dry period, when she should have been building up reserves for the next lactation. In addition, this cow contracted a severe case of mastitis shortly before parturition. Her daily production of milk, fat, total protein, and casein dropped

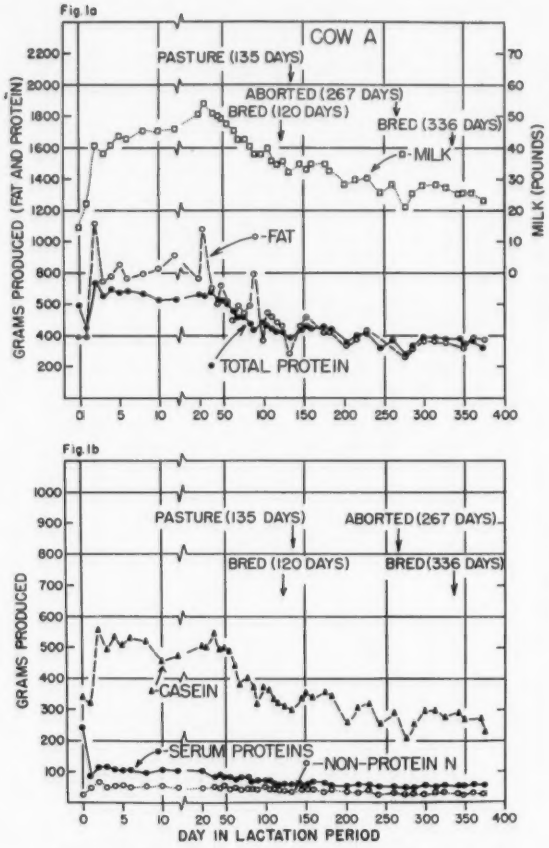

FIG. 1. Daily production of Cow A during the lactation period.

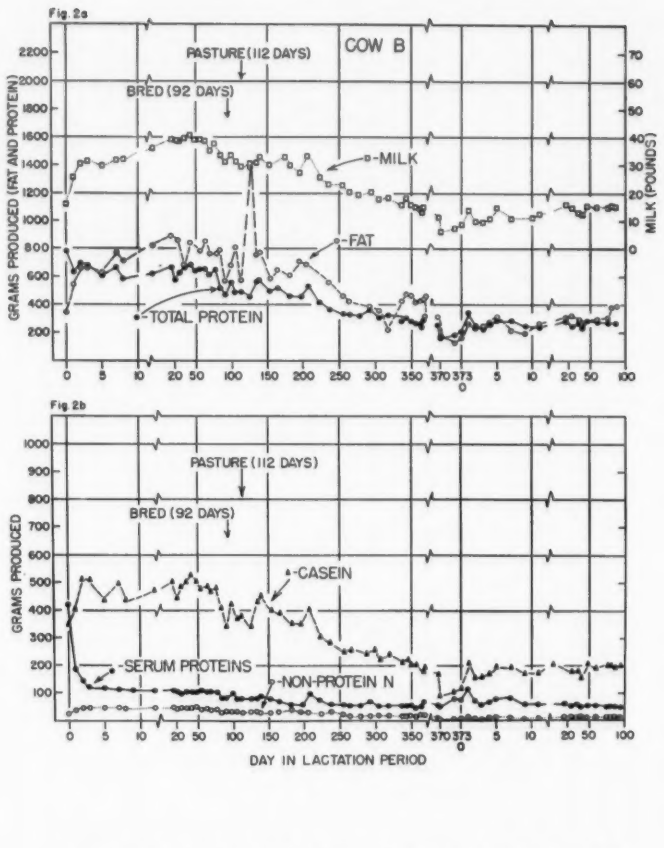

FIG. 2. Daily production of Cow B during the lactation periods.

FIG. 1a and 2a. Milk, fat, and total protein.
FIG. 1b and 2b. Casein, serum proteins, and nonprotein nitrogen.

FIG. 1b and 2b. Casein, serum proteins, and nonprotein nitrogen.

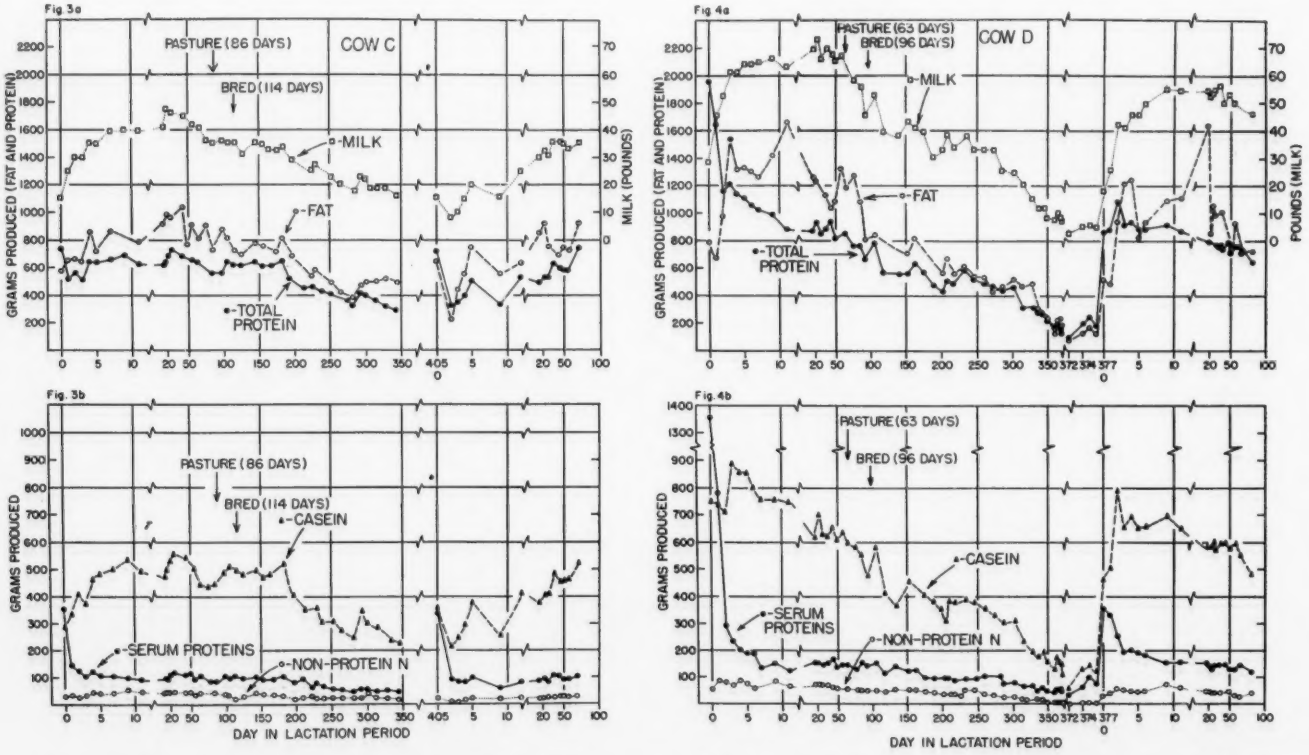

FIG. 3. Daily production of Cow C during the lactation periods.

FIG. 4. Daily production of Cow D during the lactation periods.

FIG. 3a and 4a. Milk, fat, and total protein.
FIG. 3b and 4b. Casein, serum proteins, and nonprotein nitrogen.

in the few days preceding calving, but no drop was observed in her total serum protein production. After parturition her production for all constituents assumed approximately the same level that it was in the weeks preceding it. No tendency for the protein to increase was evident, showing again that this cow was in full protein production on the day of parturition.

The production curves for Cow C in the following lactation do not reflect what was found in her first or in the lactation periods of the other cows studied in respect to protein production. Along with the increase in the daily pounds of milk and fat produced, the daily protein production also increased markedly after parturition. Cow C was over a week late in freshening and delivered a dead calf, at which time she had a high fever in addition to severe mastitis, which continued until she was removed from the experiment. The second lactation of this cow must be considered a special case and will be discussed later.

The production curves for Cow D in the following lactation may also reflect to a smaller degree the influence of milking through the dry period, since she did not quite attain the maximum production level found in the preceding lactation. Again, the production of pounds of milk and fat increased in the first month or so, whereas the total protein production was essentially at a maximum at parturition. Similar to Cow B, her production of pounds of milk, fat, and protein, with the exception of the serum proteins, dropped rapidly in the days preceding parturition.

The curves in Figures 1a, 2a, 3a, and 4a indicate that the daily protein production during the lactation period fluctuated less than the fat production. The percentage of protein in the milk decreased during the first month of lactation while the total pounds of milk produced per day was increasing. This tendency was reversed as the lactation period proceeded when the pounds of milk produced per day dropped more rapidly than did the yield of protein; hence, the percentage of protein (both casein and the serum proteins) tended to increase in the milk. These observations on the drop and rise in the protein content of milk during the lactation cycle essentially agree with the results of various investigators *(2, 10, 19, 21)*. These trends are illustrated in the data of Table 2 where the per cent composition of fat, total protein, casein, and the serum proteins is given at times during the lactation period.

The relationship of the amount of protein and fat produced per day changed considerably during the lactation periods. During the first month or two the ratio of protein to fat tended to decrease and then increase in the months following. In three of the four cows, the daily amount of fat produced fell below that of protein and in the fourth fell almost to the same level (see Figures 1a, 2a, 3a, and 4a). The ratio of protein to fat then decreased as the end of the lactation period drew closer and the relative fat production increased.

The day to day values for the ratio of protein to fat production varied considerably, as shown in Figures 1a, 2a, 3a, and 4a. In fact, examination of these figures indicate that any ratio from about 0.5 to 3 could be found during the lactation periods. Even the ratio of protein to fat produced during the 345-day calculated lactation periods by the four cows (data in Table 1) varied greatly.

TABLE 1

Data on the experimental cows

	Cow No.			
	A	B	C	D
Breed	Holstein	Guernsey	Guernsey	Holstein
Age at parturition *(mo.)*	26	89	27	83
Previous calves *(No.)*	0	5	0	4
Lactation period *(days)*	345	345	345	345
Milk yield *(lb.)*	11,500	9,760	10,610	14,900
Fat yield *(lb.)*	380	490	530	566
Av. fat *(%)*	3.3	5.0	5.0	3.8
Protein yield *(lb.)*	330	370	425	490
Av. protein *(%)*	2.9	3.8	4.0	3.3
Protein/fat	0.88	0.76	0.80	0.87

These observations prompted a look into some of the background material accumulated over many years that has led to the often-expressed feeling that there is a close relationship between the protein and fat composition in milk.

Apparently it has been too often tacitly assumed that the highly significant positive correlation between the protein and the fat content of milk allows an accurate estimation of the protein per cent from the known fat per cent. Turner *(20)*, using the data of Overman *et al. (16)*, constructed a graph showing the relationship of the protein and the fat composition of milk, which has been often

TABLE 2

Changes in the composition of fat, total protein, casein, and the serum proteins during the lactation period

Cow No.		Approximate day in lactation period[a]					
		0	20	100	200	300	330
A	Milk *(lb.)*	14.0	52.5	37.5	30.0	27.3	26.4
	Fat *(%)*	5.9	3.9	3.1	2.8	2.8	3.3
	Total protein *(%)*	9.3	2.8	2.8	3.0	3.0	3.1
	Casein *(%)*	5.4	2.2	2.1	2.3	2.3	2.4
	Serum protein *(%)*	3.7	0.42	0.42	0.43	0.47	0.51
	Total protein/fat	1.6	0.72	0.90	1.1	1.1	0.94
B	Milk *(lb.)*	16.9	38.4	31.4	28.7	18.9	16.7
	Fat *(%)*	4.6	5.1	4.9	5.1	4.3	6.1
	Total protein *(%)*	10.4	3.7	3.7	3.7	3.9	3.9
	Casein *(%)*	4.5	2.8	2.8	2.8	2.9	2.9
	Serum protein *(%)*	5.5	0.63	0.62	0.63	0.74	0.79
	Total protein/fat	2.3	0.78	0.82	0.78	0.91	0.64
C	Milk *(lb.)*	15.7	46.1	36.1	27.6	23.7	17.4
	Fat *(%)*	8.5	4.8	5.0	5.1	4.2	6.4
	Total protein *(%)*	9.5	3.5	3.9	4.1	4.1	4.4
	Casein *(%)*	4.1	2.7	3.1	3.2	3.4	3.4
	Serum protein *(%)*	5.1	0.57	0.65	0.69	0.56	0.70
	Total protein/fat	1.1	0.77	0.78	0.80	0.95	0.69
D	Milk *(lb.)*	27.4	69.7	44.6	34.0	23.9	13.0
	Fat *(%)*	6.4	3.6	3.6	3.6	4.4	5.0
	Total protein *(%)*	18.3	2.9	3.3	3.4	3.8	4.4
	Casein *(%)*	6.4	2.1	2.4	2.4	2.7	2.9
	Serum protein *(%)*	11.6	0.52	0.66	0.61	0.66	0.87
	Total protein/fat	2.9	0.81	0.92	0.94	0.86	0.88

[a] With the exception of the first day, all figures are averaged values of two to four composited daily samples taken on days on or near the designated day.

quoted *(4)*. Overman *et al. (13, 14)*, in later studies on the milk of the Brown Swiss breed found highly positive correlation coefficients between the protein and the fat composition but nevertheless concluded, "Thus it appears that knowledge of the per cent of fat in mixed herd milk from purebred Brown Swiss cows would be of little value for inferring the per cent of protein" *(14)*.

McDowall *(11)* in an extensive study of the use of milk in cheese making found that knowledge of the per cent of fat in milk was not reliable enough as an index of the total protein or casein present to be used as a basis of payment, even with milk from a specific area and time of the year. In an analysis of the relationships between protein and fat composition found by various investigators *(1, 5, 6, 15, 16, 18, 21)*, McDowall pointed out that large differences would be obtained in the predicted protein per cent of a milk based on the straight line relationship equations between protein and fat formulated by these investigators. This along with his own data prompted McDowall to conclude "—that there is no such thing as a sound average relationship between fat and total protein in milk—." Several investigators have noted that the ratio of the protein to the fat composition changes during the months of the lactation cycle, with great variations shown by different cows *(3, 10, 11, 19, 21)*. The effects of season and locality on the protein to fat ratio have been observed *(11, 12)*. Although much of the variation is undoubtedly due to breed and environmental considerations, some of it must also be due to the fact that the milk volume varies considerably during the year, since in most areas more cows freshen or reach late lactation in some months than in others. This would help to explain why there have been such different statistical evaluations of the relationship between protein and fat composition. It is well to remember that in most studies the correlation between protein and fat has been established with large numbers of accumulated data for mixed milk, which in general has been composed of more of the milk from cows in the midst of their most productive months of the lactation period. Such a correlation or graph composed on the basis of this average data would be of little or no value for inferring from the fat percentage the protein composition of a sample of milk from a cow on a specific day during the lactation period.

In general, the production studies for these cows indicate that protein production was essentially at its maximum on the day of parturition. It should not be assumed, however, that such would be the case for every lactation period studied. Of the four complete and the three partial lactations of the four cows studied, only in the second lactation of Cow C was there an increase in the protein production during the period of increasing milk yield. Cow C must be considered a special case in that during her second lactation she experienced difficulties with mastitis especially at parturition. Her sickly condition at her second calving undoubtedly reflected itself in a lowered vigor and vitality for several weeks. Studies on her blood protein levels during this time indicated a tremendous increase in immune γ- and β-globulins *(9)*. Thus, the production of Cow C should be interpreted as being under an abnormal situation. It should not be at all surprising to find that a cow which started her lactation in poor shape

through sickness or poor feeding practices and then subsequently reached a healthy, well-fed condition would increase in absolute daily protein production later in the lactation cycle.

The effects of breed, feeding practices, season, climate, etc., on the protein producing capacity of the cow would certainly need to be determined before it could be assumed as a general rule that protein production is essentially at its maximum on the day of parturition. However, it is well known that the milk yield and fat production as a general rule do reach their maximum a month or so after parturition. Since the lactation periods studied in this investigation fulfilled these two conditions and demonstrated a maximum of protein production at or near parturition, there is no reason to suspect that this may not be a general rule also.[2] This conclusion is also supported by the data of Turner *(20)* and reproduced by Espe and Smith *(4)* on the average composition of the first eight milkings of five cows after parturition. Calculation of this data to show the total protein yield in these milkings indicates that the production was highest on the day of parturition and then decreased to a rather constant output in about the fourth through the eighth milkings, during which time the production of pounds of milk and fat was increasing steadily. The results of Azarme *(2)* on the protein content of milk during the lactation period after the first week also tend to support this conclusion.

Even though the output of total protein was at a maximum at parturition, it does not necessarily mean that the cows were undergoing maximum protein synthesis at this time. Certainly for fat production these cows did not reach maximum production until a month or so after parturition. For casein production these cows attained a maximum of synthesis in but a few days. It should also be noted that the output per day of nonprotein nitrogen followed the production of casein in that its maximal output was reached in a few days. The free amino acids, purine and pyrimidine bases, urea, ammonia, etc., contained in this fraction are logically postulated to be by-products of the metabolic activities of the mammary gland. Apparently the amount of nitrogen compounds present in this fraction reflects the level of protein synthesis going on much more than the synthesis of fat or of the total pounds of milk.

During the first few days while the casein production was increasing, the simultaneous decrease in serum protein output helped to maintain the total protein level rather constant or caused it to decrease. Studies on the blood serum proteins of these and other cows *(9)* have indicated that in the few days prior to parturition some of the specific immune blood protein components decrease greatly. As much as 10 to 30% of the total serum protein content of blood may be lost at this time and is chiefly responsible for the high immune globulin level in the colostrum at parturition. These immune globulins are replaced in the blood stream by the animal during the time that the milk yield is increasing daily *(9)*. This competition between the mammary gland and the centers of immune globulin

[2] Similar studies on four additional cows of low and high production have added further confirmation. The lactation periods of these animals began in the summer months.

production would tend to reduce the resultant amount of protein appearing in the milk or the blood if nitrogen and/or the essential amino acids were in short supply. Whether the protein be synthesized for the blood or the milk, the requirements of the animal for amino acids and energy to accomplish the synthesis would be about the same. This would indicate the necessity of making available to the cow an adequate protein or nitrogen level around the time of parturition when her needs are the greatest and not increasing to a maximum a month or so later figured on the amount of fat or milk produced. In this respect, the importance of accurately determining in production studies the protein production for the first week after parturition should be noted in order to obtain an appreciation of the protein requirements of the cow at this time.

Determination of the specific casein and serum proteins produced during the lactation periods of these cows indicated that unlike total casein production, which reached a maximum in a few days, some of them were being produced at their maximum on the day of parturition *(8)*. These studies on the production of the specific casein components (α-, β-, and γ-) and the specific serum protein components (immune globulins, α-lactalbumin, β-lactoglobulin, and serum albumin) during the complete lactation periods of these cows will be reported on later.

SUMMARY

The production level of the milk proteins was evaluated during the entire lactation period of several cows and compared to the production of total milk and fat. In contrast to the normal attainment of maximum daily production of milk and fat after about the first month, the total amount of protein produced per day tended to be at a maximum on the day of parturition and either remain constant or decrease during this time. Maximum casein production was reached in but a few days; however, the simultaneous decrease in serum protein output tended to maintain the total protein production in the first few days or to decrease it. Large variations were found to occur during the lactation periods in the ratio of protein to fat production.

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METABOLISM OF BOVINE SEMEN. III. UPTAKE AND METABOLIC UTILIZATION OF GLYCEROL-1-C^{14} BY BOVINE SPERMATOZOA[1,2]

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The addition of glycerol to diluted bull semen enables a large percentage of the spermatozoa to survive freezing and storage at –79° C. *(14)*. Many substances *(2, 15, 16)* have been investigated for a similar protective action, but none as effective as glycerol has been found.

Several theories *(6-11, 17)* have been advanced as to the mechanism of the protective action of glycerol for tissues, blood cells, and spermatozoa frozen to low temperatures. Although several workers *(8, 13, 18)* have suggested that glycerol enters spermatozoa, there is no direct evidence to show that this action takes place. Conflicting evidence exists as to the ability of bull spermatozoa to oxidize glycerol. Mann *(12)* reported that glycerol is not oxidized by bull spermatozoa. On the other hand, White *et al. (18)* found that the addition of glycerol to the substrate containing bull or ram spermatozoa resulted in an increase in lactic acid production above that obtained when no glycerol was present. The present study was designed to determine *(a)* whether glycerol enters bull spermatozoa and *(b)* whether glycerol is capable of being catabolized by bull spermatozoa.

EXPERIMENTAL PROCEDURE

Semen used in these experiments was collected from Guernsey and Holstein bulls by means of the artificial vagina. Only semen samples having an initial motility of 60% or higher and a concentration of at least 750×10^6 spermatozoa were used. The volume of diluted semen used in each experiment varied but total numbers of spermatozoa were held constant. Thus several ejaculates were pooled to provide a total of three billion spermatozoa in the diluted semen. The semen was diluted initially to one-half the final volume and cooled to 5° C. in 1 to 2 hours. The semen was then diluted to final volume by adding, in three portions at 10-minute intervals, an equal volume of diluter containing sufficient labeled glycerol to give a total radioactivity of 10 μc. On a volume basis this gave a final concentration of from 6.5 to 12.5% glycerol. One thousand units of penicillin and streptomycin were added per milliliter of glycerolated semen. Except in Experiment 1, the semen samples were permitted to equilibrate overnight (18 to 20 hours) under nitrogen at 5° C. The incubation flasks were similar to those described by Bloom *et al. (1)*. After equilibration the carbon

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dioxide in the flask was recovered and assayed for radioactivity by the method described by Flipse and Almquist *(4)*.

The efficiency of the washing procedure *(3)* was compared in all experiments except Experiment 4 by using three identical flasks. The spermatozoa in flask 1 were washed three times by centrifugation and resuspension in 15 ml. of physiological saline containing 10% by volume of unlabeled glycerol. Those in flask 2 were washed four times and those in flask 3, five times. After final centrifugation, the spermatozoa were suspended in 0.5 ml. of 0.9% NaCl solution containing 10% glycerol. A 0.5-ml. portion of this suspension was transferred by a micropipette to a 1⅛-in. aluminum planchet. A small disk of lens-cleaning tissue was placed on top of the suspension to obtain uniform spreading, and the samples were dried under an infrared lamp. All samples were assayed for radioactivity in a windowless flow gas G.M. counter in conjunction with a decade scaling unit. Radioactivities of the CO_2 samples were corrected for self-absorption to a constant thickness of 1 mg. of barium carbonate per square centimeter.

RESULTS AND DISCUSSION

Experiment 1. In this experiment an attempt was made to duplicate the processing procedure followed in the preparation of semen for freezing. The semen samples were diluted in fresh pasteurized skimmilk obtained from the University Creamery. The skimmilk was heated to 92° C. and held at this temperature for 10 minutes before being used as a diluter. As shown in Table 1,

TABLE 1

Effect of post-incubation washing upon the radioactivity of spermatozoa incubated for 18 to 20 hours at 5° C. with glycerol-1-C^{14}

Experiment	Spermatozoa washed		
	3 times	4 times	5 times
	(c.p.m.) [a]	*(c.p.m.)*	*(c.p.m.)*
1	81	29	53
2	534	141	162
3	206	96	95
4		957	

[a] Counts per minute per 2×10^9 spermatozoa.

low radioactivities were obtained for the sperm suspensions after incubation for 18 to 20 hours at 5° C. in heated skimmilk containing 10% by volume of glycerol-1-C^{14} (10 μc.). Since skimmilk contains substances which might be capable of entering the sperm cell, it is possible that a competitive situation exists, thus preventing a maximum permeation of the cells by glycerol.

Experiments 2 and 3. In Experiment 1 it was noticed that only a relatively small percentage of the total radioactivity originally present was recovered. This suggested that a considerable amount of the glycerol might have been oxidized by the spermatozoa with a resulting loss of C^{14} as carbon dioxide. Thus, in the present experiments physiological saline was used as the diluent and the samples were incubated under nitrogen by using flasks designed to

TABLE 2

Specific activities of barium carbonate prepared from the carbon dioxide liberated from preparations incubated under nitrogen for 18 to 20 hours at 5° C. with glycerol-1-C¹⁴

Experiment	Radioactivity in CO_2 from		
	Semen	Spermatozoa	Seminal plasma
	(c.p.m. per mg. $BaCO_3$)		
2	3120[a]		
3	910[a]		
4		4740	24

[a] Average of three aliquots.

trap the CO_2 given off during incubation. The results of incubating bovine spermatozoa diluted in 0.9% NaCl solution containing 6.5 to 7.0% by volume of radioactive glycerol (10 μc.) are shown in Tables 1 and 2. The sperm suspensions in these experiments showed much more activity than those obtained in Experiment 1. These results show that glycerol definitely enters the sperm cell. Obviously, more glycerol enters the sperm cells when they are suspended in physiological saline than when they are diluted in heated skimmilk. As shown in Table 1, a minimum of four washings is needed to remove all of the adsorbed glycerol from the sperm cells.

The specific activities of CO_2 given off by spermatozoa incubated under nitrogen for 18 to 20 hours at 5° C. with glycerol-1-C¹⁴ are shown in Table 2. At the present time there is no satisfactory explanation for the higher counts obtained in Experiment 2. In spite of the variation between the counts obtained in the two experiments, the counts in Experiment 3 are still large enough to show that glycerol is oxidized by diluted bull semen.

Experiment 4. To determine the separate effects of the ability of seminal plasma and spermatozoa to oxidize glycerol, the sperm cells were separated from the seminal plasma and both fractions were incubated with glycerol-1-C¹⁴. After separation by centrifugation, the sperm cells were resuspended in heated skimmilk diluter and the seminal plasma was added to a 0.9% NaCl solution, both of which contained 10 μc. of glycerol-1-C¹⁴. In addition to determining the specific activities of CO_2 from the spermatozoa and seminal plasma mixtures, the sperm cells also were washed four times and assayed for radioactivity. From the results presented in Table 2 it can be seen that the oxidation of glycerol is due almost entirely to the metabolic processes of the spermatozoa. The very low activity in the CO_2 obtained from the seminal plasma mixture probably was due to the oxidative metabolism of glycerol by a small number of sperm cells that were not removed from the seminal plasma by centrifugation.

Upon assaying the spermatozoa for radioactivity it was found that the activity was almost seven times as great as that obtained from the sperm suspensions in Experiments 2 and 3. A part of this apparent increased uptake may be due to the absence of seminal plasma. Flipse *(3)* has shown that removal of seminal plasma either enhances uptake or reduces catabolism of adsorbed nutrients by bull spermatozoa.

The data presented confirm the results reported by White *et al. (18)* that glycerol is metabolized by bull spermatozoa. However, they do not indicate the

pathway involved. In bacterial preparations, at least two pathways for glycerol metabolism have been reported. Wiame *et al. (19)* found that glycerol was converted to dihydroxyacetone which then was phosphorylated and passed through the triosephosphate reactions to pyruvate and the Krebs cycle. On the other hand, Hauge *et al. (5)* report that in *Acetobacter suboxydans* the dihydroxyacetone phosphate undergoes condensation to produce fructose-1,6-diphosphate, which eventually is degraded via the pentose cycle. Either pathway could account for the rapid turnover to carbon dioxide observed in semen preparations. Additional research is necessary to establish the point at which glycerol enters the cycle and the pathway involved in its metabolism.

SUMMARY

It has been shown that glycerol-1-C^{14} enters bull spermatozoa in measurable amounts and is converted to $C^{14}O_2$ during anaerobic incubation. The oxidation of glycerol was shown to be due to the metabolic processes of the spermatozoa and not to enzymatic activity of seminal plasma. Incubation of sperm, which had been separated from the seminal plasma, in the presence of glycerol-1-C^{14} showed high radioactivity in the CO_2 given off, whereas there was relatively no activity present in the CO_2 collected from seminal plasma incubated with glycerol-1-C^{14}.

Bull spermatozoa which had been separated from the seminal plasma showed a higher uptake and a higher metabolic utilization of glycerol than did spermatozoa in the presence of seminal plasma.

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TECHNICAL NOTES

DEVELOPMENT OF OXIDIZED FLAVORS IN UNHOMOGENIZED AND HOMOGENIZED MILK

Samples of unhomogenized and homogenized milks were obtained from the laboratory of the Department of Dairy Industry during the winter feeding season extending from Dec. 20, 1954, to May 14, 1955. They were taken directly from the bottler and held at 36° F.

The oxidized flavors scoring system was: –, no oxidized flavors; ?, indication of oxidized flavors; 1, slightly oxidized flavors; 2, distinctly oxidized flavors; 3, strongly oxidized flavors; and 4, very strongly oxidized flavors. It is considered that a score of 2 would be complaint milk by the most critical consumers and that the average person would not be satisfied with milk that scored 3. The milks were scored on the 3rd, 5th, 7th, 10th, and 14th days.

The following figures showing the scores in February, 1955, are representative of those of the other months and are a good summary of the study.

E. S. GUTHRIE
Cornell University, Ithaca, New York

Feb. 1955	Unhomogenized					Homogenized				
	3d	5d	7d	10d	14d	3d	5d	7d	10d	14d
1	1	1	1	2	2	–	–	–	–	–
2	–	?	2	3	3	–	–	–	–	–
3	2	3	3	3	3	–	–	?	?	–
4	–	1	2	3	2	–	–	–	–	–
5	?	2	3	3	3	–	–	?	–	–
7	?	3	3	3	3	–	–	–	–	–
8	?	1	1	2	2	–	?	–	–	–
9	–	–	?	2	1	–	–	–	–	–
10	–	–	2	2	2	–	–	–	–	–
11	–	1	2	3	3	–	?	–	–	–
12	.....					–	–	–	–	–
14	–	2	3	2	3	–	–	–	–	–
15	–	–	?	2	3	–	–	–	–	–
16	–	2	1	1	3	–	–	–	?	?
17	–	?	2	2	2	–	?	–	?	–
18	–	–	–	?	–	–	–	?	?	–
19	.....					–	–	–	–	–
21	–	3	3	3	3	–	–	?	–	–
22	–	–	–	–	1	–	–	–	–	–
23	–	–	–	1	1	–	–	–	–	–
24	–	3	2	2	2	–	–	–	–	–
25	–	–	?	–	1	–	–	–	–	–
26	–	–	?	2	1	–	–	–	–	–
28	–	3	3	3	3	–	?	–	–	–

APPLYING HILLIG'S RAPID METHOD FOR WATER-INSOLUBLE ACIDS TO FRESH MILK[1]

The water-insoluble fatty acid content of farm-separated cream has been a subject of investigation at this station since early 1951 *(1, 2)*. Results of the work show that method of separation, efficiency of cooling and storage temperature on the farm, age of cream when delivered to buying station, temperature at which cream is held from time of purchase until churned, season of year, and fat content of cream are all related to the water-insoluble acid (WIA) content of the cream.

It was believed that an explanation of the pronounced seasonal trend in WIA content of farm-separated cream would not be forthcoming until more data were available relative to

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published with the permission of the director.

the basic circumstances affecting the liberation (or production) of water-insoluble free fatty acids in milk and cream. Accordingly, a project was begun in April, 1955, to study conditions influencing the WIA content of fresh individual cow's milk.

Briefly, the technique consisted of separating mechanically the milk of an individual cow within approximately 15 minutes of the time it was drawn. An analysis of the WIA content of the resulting cream was made immediately by Hillig's rapid method *(3)*. This procedure made it possible to obtain WIA values on all cream samples within 1 to 2 hours of milking. It was believed that such data would represent, essentially, the original WIA content of the milk as secreted by the cow.

After several preliminary trials were conducted, it was observed that, even when following the above procedure, WIA values continued to be roughly proportional (inversely) to the fat content of the cream. It was further discovered that the WIA value of the cream separated from a given lot of fresh raw milk could be varied within relatively wide limits simply by changing the setting on the skimmilk screw. This unexpected phenomenon was demonstrated in a slightly different fashion by the following procedure.

WIA determinations were made on three portions of the same lot of freshly separated raw cream as follows: *(a)* cream as separated, *(b)* cream diluted with an equal weight of skimmilk (same source as the cream), *(c)* cream diluted with an equal weight of distilled water. Typical results obtained are shown in Table 1.

TABLE 1

	WIA	
	Jersey	Holstein
Cream	70	72
Cream and skim	129	94
Cream and H_2O	82	84

The observations thus far noted indicate that during the interval between milking and titration of the extracted WIA (never more than 2 hours), a certain amount of lipolysis occurred. This would seem to be explained most logically on the basis of the agitation to which the fat was subjected at two points: *(a)* during mechanical separation of the milk and *(b)* during "churning" of the sample in the analytical procedure. This explanation harmonizes with the observations of Krukovsky and Sharp *(4, 5)* that agitation of raw milk "activates" the lipase in the milk.

As further supporting evidence for the foregoing explanation, it was observed that the low-fat cream samples usually required a much longer "churning" period, thereby increasing the amount of agitation to which the fat was subjected. Pfeffer *et al. (6)* stated that the "lipolytic factor" in milk is mostly in the serum portion. This is probably the reason for the greater increase in WIA when the cream was diluted with skimmilk than when diluted with water.

Data obtained under the circumstances described above would be of little worth for evaluating the possible effect of various biological and environmental conditions on the WIA content of freshly drawn milk, or for establishing standards for this constituent. Seemingly it would be necessary to inactivate the lipase immediately after the milking operation. It was thought that heat treatment would be the most practical solution to the problem. Heat treatments investigated and typical WIA values obtained on the resulting creams are shown in Table 2.

TABLE 2

	WIA values of cream		
		diluted with	
Heat treatment of milk	Undiluted	Equal wt. of skim	Equal wt. of H_2O
145° F. for 30 min.	61	87	82
150° F. for 30 min.	54	81	65
160° F. for 30 min.	45	47	46

The writers consider that the method wherein the milk is held at 160° F. for 30 minutes yields results that are truly indicative of the WIA concentration in the freshly drawn milk. It is standard procedure at the Kentucky station when performing WIA assays on fresh raw milk.

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PEOPLE and EVENTS
in the Dairy Science World

Pioneers in the Dairy Industry

The retirement of FRANK BARRON MORRISON to emeritus rank ended almost 45 years of active leadership in the fields of animal husbandry and livestock nutrition. His books, *Feeds and Feeding* and *Feeds and Feeding, Abridged,* not only are standard text and reference books for high school and college courses in animal husbandry but also are widely used by livestock farmers and in the feed industry. Farmers and stockmen have been influenced through his writings in the popular farm press and in scientific publications and also through his radio talks and many appearances on farmers' institutes and farm and home week programs in practically every state in the Union. His services have been sought as a consultant by a number of foreign countries, where he has made surveys for the governments or other agencies. Among these countries are the Philippines, Germany, Argentina, and Venezuela.

F. B. Morrison

Dr. Morrison was born at Fort Atkinson, Wis., on May 19, 1887, the son of Scotch-English farm folk. His parents moved to a farm near Marshfield while he was still a young boy. After graduation from the Marshfield High School, where he was active in debating, he taught a country school for 3 years before entering the Univ. of Wisconsin in 1907. He majored in agricultural chemistry and was graduated with highest honors in 1911.

It was during this period that he first became associated with DR. W. A. HENRY, then dean emeritus of the Wisconsin College of Agriculture. Dean Henry had published the original edition of the book, *Feeds and Feeding,* in 1898. It met with immediate success and soon became the outstanding textbook in its field. When in 1909 a revision became necessary, Dean Henry was in failing health. He therefore sought the services of a graduate student or senior to help in the tedious work of compiling data. At this time young Morrison was only a sophomore, but he badly needed money to remain in college. He applied for the job before anyone else, and he got it.

After graduation, Morrison was appointed administrative asistant to DEAN H. L. RUSSELL of the College of Agriculture. At the same time he held an assistantship in the Dept. of Agricultural Chemistry and carried on graduate work. In 1912 he was appointed an instructor in agricultural chemistry, meanwhile continuing his other duties. In 1914 he was promoted to assistant professor of animal husbandry and was asked to take charge of investigations in livestock feeding. During this period he began to develop an interest in the application of nutritional chemistry to the feeding of farm animals.

In 1919 he was promoted to a full professorship. Recognition of his ability as an administrator, research worker, and lecturer resulted in an appointment as assistant director of the Agricultural Experiment Station, which position he held until 1927. During 1925 to 1926 he was acting dean and director while Dean Russell was in the Orient.

While Professor Morrison was advancing in the academic world, he was also working on the revision of both *Feeds and Feeding* and *Feeds and Feeding, Abridged,* and in 1917 a condensed version of both books appeared under the co-authorship of W. A. Henry and F. B. Morrison. This book, primarily intended for use in high schools and vocational schools, filled a long-felt need for a simplified textbook in animal husbandry and livestock feeding. Upon Dean Henry's death in 1932, Dr. Morrison became the sole author of both books. Constant changes and recent discoveries in the fields of animal nutrition and animal husbandry have required periodic revisions of these standard texts. The 21st edition of *Feeds and Feeding* was published in 1948, and the 8th edition of *Feeds and Feeding, Abridged,* in 1949.

On Nov. 24, 1910, Dr. Morrison was married to Elsie Rea Bullard, a fellow student at the Univ. of Wisconsin and a graduate of the School of Journalism. Her training enabled her to be of great assistance in each of the successive revisions of the books. She also assumed management of the publishing company in 1915 and still continues in this capacity.

In 1927 Dr. Morrison was made director of the New York State Agricultural Experiment Station at Geneva, N. Y., and of the Cornell Univ. Agricultural Experiment Station at Ithaca, N. Y. He remained in this position until he became head of the Animal Husbandry Dept. at Cornell Univ. in 1928.

The Univ. of Vermont awarded him the honorary degree of Doctor of Science in 1947, and the Univ. of Wisconsin awarded him the same degree in 1950. He is a member of numerous scientific and honorary societies and organizations. For many years he has been active in the First Presbyterian Church of Ithaca, being an elder and having served several times as chairman of the board of trustees.

In 1946 Professor and Mrs. Morrison together established an annual award, later made perpetual through a trust fund, which enables the American Society of Animal Production to give a substantial cash prize each year to a scientist selected by the Society for his outstanding research work in livestock production. In 1950 they also established an endowed fellowship at Cornell Univ. to foster the training of graduate students, especially those from foreign lands, in the field of livestock feeding.

Although June, 1955, marked the end of an active academic career for Dr. Morrison, all who know him well realize that other interests and pursuits will continue to claim his attention. His recent purchase of a mountain retreat on the shore of Upper Saranac Lake, N. Y., will afford him the quiet and leisure for continued writing and for keeping his famous books up-to-date.

Kirchoff Meets Tragic Death

GEORGE E. KIRCHOFF, 56, for many years a member of the Jefferson County (Alabama) Health Department, was killed Dec. 15 when struck by a car near his home in Birmingham. Since 1944 Mr. Kirchoff has been director of the food and dairy division of the county Health Department. He was recuperating from a brain operation at the time of the accident. A native of Iowa, Mr. Kirchoff went to Birmingham after graduation from Iowa State in 1923. He is survived by his widow and two children.

Dairy Remembrance Fund Graduate Assistance Grant Available

Dairy Remembrance Fund, Inc., has one $500 Graduate Student Assistance Grant available for a promising and worthy student pursuing graduate study in the dairy production or manufacturing field. Those who have students in particular need of supplementary funds for the completion of degree work are requested to write the chairman of the Allocations Committee, W. A. WENTWORTH, The Borden Company, 350 Madison Ave., New York 17, for further information and application forms.

The Farmer's Share of Consumer's Dollar[1]

There is much talk about the ever diminishing share of the consumer's dollar that the farmer receives. All will admit that is the trend without getting out the record books or studying complicated charts. The farmer receives less and less of the consumer's dollar each year. We can begin with that premise.

Why is it so? Isn't that answer easily found? Think back only a short time and we see how rapidly the food products that go into the average kitchen have changed. How much farther the foods are removed from the form in which they were when they left the farm as raw products.

Some of us older people can remember when the neighbor came to the farm and bought eggs fresh from the nest and he carried them home in the pail he brought with him. There was no middleman, nor was there any manufacturing process between the farmer and the consumer. The farmer got 100% of the consumer's dollar.

Neighbors brought their pails to the dairy barn and the milk was poured direct from the strainer pail, as fresh as it was drawn a minute before from the cow's udder, directly into the neighbor's pail. It was warm, sweet, and fresh. True, it cost the consumer only four cents for a liberal quart but the farmer got 100% of that consumer's pennies spent for milk.

Years ago when cellars were large and fit for vegetable and fruit storage, bushels of all the well known varieties of apples were carried there by the consumer direct from the orchard where he perhaps picked them from the tree. Whatever cash may have been paid for them the farmer got 100% of the consumer cash outlay for apples.

And so it was with potatoes, other vegetables, pork, beef, and chickens direct from the roosts with feathers on. And so it was with other foods, the farmer getting 100% of the consumer's food dollars. Then there was no cold storage charge, there was no processing charge, there was no delivery charge. There were few foods from far away places. How times have changed. Think of the change in service that is now between the raw products of the farm and the fancy small packages in which the processed products from distant lands and factories that come daily to the kitchen. Think how much farther the source of foods (the near-by farms) have been removed from the modern kitchen. Think how many middlemen and how many processes have moved in between consumer and farmer.

This is November—the month for election. Sensational catch phrases are flying around. The old one, that consumers' dollar and the farmers' share of it, is ever around. Let us look at that one and always remember that someone pays for all the services between the original products and finished product as it is bought by the consumer. The trend is there. It will not be changed by farmers nor by politicians. The consumer wants the service and he pays for it. Don't let anyone "kid" you about that.

[1] Reprinted from Metropolitan Milk Producers' News—November, 1955.

Cooperative Graduate Summer Sessions in Statistics

The Univ. of Florida, North Carolina State College, Virginia Polytechnic Institute and the Southern Regional Education Board are jointly sponsoring a series of cooperative summer sessions in statistics.

The third of these summer sessions will be held at North Carolina State College, June 11–July 20, 1956. A session is scheduled to be held at Virginia Polytechnic Institute in 1957 and at the Univ. of Florida in 1958. Each summer session lasts 6 weeks and each course carries approximately three semester hours of graduate credit.

The 1956 session will be held jointly with the Institute in quantitative research methods in agricultural economics, sponsored by the Social Science Research Council. Several statistics courses will be oriented towards economic applications.

The combined faculty for the 1956 summer session and Institute at North Carolina State College will include: R. L. ANDERSON, GERTRUDE M. COX, A. L. FINKNER, A. H. E. GRANDAGE, R. J. HADER, CLEON HARRELL, JACK LEVINE, and R. J. MONROE, all of N. C. State College; D. B. DUNCAN, Univ. of Florida; E. O. HEADY, Iowa State College; C. G. HILDRETH, Michigan State Univ.; and W. L. SMITH, Univ. of North Carolina.

Courses to be offered this summer are: Statistical Methods I, Statistical Methods II (Design of Experiments), Statistical Theory I (Probability and Parent Distribution), Statistical Theory II (Sampling Distributions and Inference), Sample Survey Designs, Advanced Analysis II, Advanced Calculus for Statistics, Stochastic Processes, Econometric Methods and Linear Programming. Lectures on Linear Equations (Matrix Algebra) and Production Functions will be given in the Institute program.

Inquiries should be addressed to: PROFESSOR J. A. RIGNEY, Dept. of Experimental Statistics, North Carolina State College, Raleigh.

Wisconsin to Hold Industry Conference

The annual Dairy Manufacturers' Conference of the Dept. of Dairy and Food Industries is scheduled for March 13 and 14, 1956. Sessions on the first day are scheduled to discuss problems of marketing Wisconsin milk, including consideration of fresh concentrated milk as a possible outlet for Wisconsin milk. On the second day there will be programs on sanitation and technical control, frozen desserts, and butter and dried milks.

Authorities from both industry and universities will share their ideas, experiences, and research findings with those who attend. There will be a joint dinner meeting the first evening with the Wisconsin Dairy Technology Society.

Australian Pasture and Research Tour Being Planned

The Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization of Australia, is planning an organized tour covering the eastern pastoral areas of Australia and research centers following the Seventh Intern. Grassland Congress, which is to be held in New Zealand in November, 1956. If sufficient interest is shown, the tour will last 10 or 11 days and include such important pasture areas as those near Brisbane, Armidale, Sydney, Canberra, Deniliquin, and Melbourne. The cost, including accommodations, will be about 35 pounds Australian (about $100) although transportation costs may be subsidized, in which event the cost might be reduced to 20 pounds ($56). Anyone interested should write to: R. M. MOORE, Division of Plant Industry, P. O. Box 109 City, Canberra A.C.T., Australia.

Dairy Products Improvement Institute Meets

The ninth annual meeting of the Dairy Products Improvement Institute, Inc., was held Feb. 16 in New York, according to A. C. DAHLBERG, secretary. The session was devoted largely to a panel discussion of "Requirements for the Sanitary Production of Sweet Cream, and Milk for Manufacturing." W. A. WENTWORTH of the Borden Co., New York, is president of the Institute.

Milk Carton Council Organized

The Milk Carton Quality Preforming Council has been organized by a group of manufacturers of preformed milk cartons for the purpose of maintaining a quality control program in this field. One of the important functions of the MCQP is to act as a clearing house for the dissemination of new ideas and methods in the field of milk carton preforming.

The phenomenal increase in the use of preformed cartons, in the last 5 years, has developed from a desire of dairy plants of all sizes to package their milk products in single-service containers. The MCQP is at present engaged in preparing a code for proper operation of plants preforming milk cartons. The code is to be patterned after the requirements of the U. S. Public Health Service and state and local control agencies. In addition, the MCQP is developing standards for preformed cartons of the Pure-Pak type.

Meetings will be held semi-annually, at which time technical problems pertaining to paper board, carton forming, carton filling, testing, labeling, storage, bacteriological sampling, etc., are to be discussed. Membership is open to all segments of the industry interested in milk carton preforming and filling. Headquarters for the MCQP are at 228 N. LaSalle St., Chicago. HAROLD WAINESS has been appointed secretary.

Arizona Holds Dairymen's Conference

The fifth annual Dairymen's Conference was held at Arizona State College, Tempe, Jan. 3 and 4, 1956. The six sessions of the conference centered around the theme of Milking Dairy Cows.

Included in the program were discussions on cleaning the milking equipment, feeding for best production, the structure and functions of the udder, mechanics of milking, and udder health. A panel in discussing milker-labor management pointed up the problem of getting and keeping a milker qualified for the highly technical job of milking daily 100-150 cows.

E. GRANT MOODY conducted the program with VEARL R. SMITH, Univ. of Wisconsin, the principal speaker.

Jacobson Joins Kentucky Staff

D. R. JACOBSON on Feb. 1 assumed the duties of assistant professor in dairy husbandry at the Univ. of Kentucky. He will be in charge of dairy cattle nutrition and physiology research and will teach classes in dairy husbandry at both the undergraduate and the graduate level. Dr. Jacobson received both the B.S. and M.S. degrees at Kansas State College. He was granted the Ph.D. degree at the Univ. of Maryland and served on the staff there for 3½ years.

The position being filled by Dr. Jacobson was formerly held by C. A. LASSITER, who recently joined the dairy husbandry staff at Michigan State Univ.

Merit Award Given J. W. Bartlett

J. W. BARTLETT, head of the Dairy Dept. at Rutgers Univ. for 35 years, was given the 1956 Award for Merit of the New Jersey Milk Industry Assoc. The award, which included a silver bowl and citation, was made at the annual Farmers Week dairy banquet on Jan. 26. This is the seventh such award to be given by the Milk Industry Assoc.

In conferring the award, A. M. WOODWARD, Newark, president of the Association, praised Dr. Bartlett for 40 years of service to the dairy industry of New Jersey as "scientist, teacher, and industry leader." Mr. Woodward pointed out that "as director of the Experiment Station's 1,300-acre dairy research farm in Sussex County, Dr. Bartlett has built one of the most distinguished herds of dairy cattle in America."

DISA to Hold Annual Meeting

A lottery will be held during the regular annual meeting of the Dairy Industries Supply Assoc., March 1-2, at the Hotel Commodore in New York City to determine display locations for the Exposition which will be held Oct. 29–Nov. 3, in Atlantic City. The lottery works this way: Two empty goldfish bowls are placed on a table; in one are dropped capsules which contain, for example, the numbers of all such booths as are to have an extent of approximately 100 sq. ft.; into the other are put capsules which contain the names of companies desiring booths of this approximate size. The contents of each bowl are mixed thoroughly, and then two selected members who have had nothing to do with preparing the opaque capsules make simultaneous drawings from each receptacle. Thus are the names of companies matched up with booth numbers.

The lottery continues then, group by group, according to the participants' desired extents of area. A few exhibiting firms can not take part in the lottery because of special dimensional or weight factors in their products or because the products require utilities not everywhere accessible. Such firms simply accept suitable assigned spots for their displays.

The lottery for exhibit locations will be merely one feature of a significant annual meeting, planned by DISA, the spokesman said. Other highlights of the sessions include unusual speakers from the world of finance, reports from market surveying and market data development committees of DISA, and an election of six directors for 3-year terms to an 18-man board of directors.

National Dairy Forms Subsidiary

Sheffield Chemical Co., Inc., Norwich, N. Y., became a separate operating division of Sheffield Farms Co., Inc., as of Jan. 1, 1956, and will be known as Sheffield Chemical, Division of Sheffield Farms Co., Inc. Sheffield Farms Co., Inc. is a subsidiary of National Dairy Products Corp. The main office of Sheffield Chemical will continue to be in Norwich.

Sheffield Chemical will continue to produce and sell products derived from milk, with emphasis on highly refined and edible items for pharmaceutical, food, and biochemical applications.

J. R. BORST, who was vice-president of the Chemical Co., becomes general manager of this new division. F. A. BALDAUSKI, formerly the technical director of the subsidiary company, has been appointed sales manager and G. C. HOOKS, technical director of Sheffield Chemical. P. M. REED and A. A. SULLIVAN have been appointed production manager and operations manager, respectively, of the new division.

Dairypak to Build Addition

Dairypak Inc., a subsidiary of the Champion Paper Co. of Hamilton, Ohio, has announced plans for building a million dollar addition to its factory at Cleveland. Dairypak manufactures paper containers for dairy and food industries.

Comfort Is New Borden President

The Borden Co. has announced the election of T. G. MONTAGUE as chairman of the board and H. W. COMFORT as president. Mr. Comfort

started with the Reid Ice Cream Co., Brooklyn, N. Y., in 1918. When this company was taken over by the Borden Co. in 1928, he was the sales manager and in 1931 he became president. In 1944 he was elected executive vice-president of the Borden Co.

Mr. Montague entered the industry in 1919 as a clerk for the Valecia Evaporated Milk Co. at Madison, Wis. In 1926 he became its president and the following year he was made president of the Kennedy Milk Co. of Madison, which was purchased by the Borden Co. the following year. In 1934 he went to New York as general manager of fluid milk operations. In 1937 he was elevated to the position of president.

California Holds Dairy Conference

The annual Dairy Industry Conference was held at Davis, Calif., Jan. 23-25, 1956, with an attendance of 400 people. H. A. BENDIXEN, State College of Washington, the faculty guest speaker at the Conference, gave a series of lectures. Numerous commercial representatives and public officials cooperated in the presentation of the program, including R. W. MOJONNIER, Mojonnier Bros. Co., Chicago, who spoke on "Automation in the Dairy Industry"; G. B. REUHL, American Dairy Assoc. of California, who spoke on "New Developments in Sales Promotion"; and S. L. DENNING, Foremost Dairies, Inc., San Francisco, whose topic was "Where Dairy Products Are Sold." Mr. Denning was a member of the Dairy Industry faculty at Davis in 1918.

News Items from Oregon

G. H. WILSTER was awarded a 1-month lectureship during January by the Information Center, Division of U. S. Foreign Service, Bad Godesberg, Germany. The lectures were given at agricultural colleges and dairy plants throughout Germany and Austria. He lectured on the various phases of the dairy industry in the United States. Dr. Wilster is on sabbatical leave, having received a Fulbright lectureship last June for a year in Denmark. Mrs. Wilster accompanied him.

"Better Quality—Bigger Sales" was the theme of the 45th annual short course and convention of the Oregon Dairy Industries in Corvallis, Feb. 13-16, 1956. Ten out-of-state speakers appeared on the convention program, including, W. B. COMBS of Minnesota, J. C. BOYD of Idaho, and C. J. BABCOCK of Washington, D. C.

The American Dairy Assoc. of Oregon has recently been formed by milk producers from all areas of the state with VICTOR BIRDSEYE, Medford, elected president. The new association will work closely with the Oregon Dairy Council and the Oregon Dairy Products Commission in building consumer demands for dairy products in the state. GLENN LAY, executive secretary for the Oregon Dairy Products Commission and manager of the Oregon Dairy Council, will act as manager for the new group and will closely coordinate the work of the three organizations.

Industry Meetings at Virginia

The Dept. of Dairy Science at Virginia Polytechnic Institute has announced two spring industry meetings. A conference and clinic on cottage cheese and buttermilk manufacture was held Feb. 21-22, and a dairy laboratory technicians' short course will be held March 19-31. No previous experience is necessary to register for the course. P. M. LARGE of the Dairy Science Dept. is in charge.

Completed Theses

M.S. Degree:

R. C. DUTRA—The origin of the carbon dioxide produced in the browning reaction of evaporated milk. Univ. of California, Davis.

Ph.D. Degree:

E. N. FRANKEL—The specificity of lipase action in milk. Univ. of California, Davis.

STUDENT CHAPTER NEWS

A section devoted to the activities of dairy students

Michigan State Dairy Club News

The annual Dairy Staff-Club mixer was held Nov. 17 at the Judging Pavilion with over 50 persons attending. The mixer culminated initiation week for 15 new Club members with an impressive initiation ceremony. The remainder of the evening was spent judging dairy cows and products and participating in sports activities.

The second annual Christmas cheese sale was even more successful than the previous one. Publicity was limited to campus publications. Over 120 orders were taken, grossing in excess of $525. With Michigan cheeses available next year from the new plant, a much expanded sales program is anticipated.

Rutgers Dairy Science Club Sponsors Contest

The Rutgers Dairy Science Club sponsored its annual Fitting and Showmanship contest during December. The event was a great success with 18 students taking part. The students fit and show animals provided by the Dairy Department and chosen by drawing numbers. The animals were judged by a prominent 4-H judge. The prizes for the individual winners were donated by the various breed associations. Another prize, the Bartlett Trophy, is awarded to the highest team—4-year students or short course students. The trophy was won by the short course students.

The Club mailed out its annual Christmas Letter in December. This is a letter describing the activities of the Dairy Dept. and Dairy Club. It is sent to all current and former members.

Survey Made of Virginia Dare Award Winners

The results of a survey regarding the present activities of Virginia Dare award winners have been reported by Lincoln R. Young, vice-president of the Virginia Dare Extract Co., Inc., New York City. Since the inception of the awards program in 1950, there have been 125 recipients of this award from among 34 colleges. The award is presented annually to an outstanding senior in dairy technology from each of the colleges participating in the awards program. Nominees for the award are selected on the basis of "excellence in scholastic achievement and its practical application in flavor discrimination and ice cream manufacture."

Fifty-four of the 125 recipients are actively associated with the dairy industry. These are classified as follows:

35—Management and production (ice cream)
4—Trainee (ice cream)
3—Sales work (ice cream)
5—Quality control (ice cream)
4—Regulatory work
3—One each, specializing in cheese, cream, and butter, and emulsifiers and stabilizers

Following is a summary of the current activities of the entire group:

54—actively engaged in the dairy industry
34—serving in the Armed Forces
29—continuing further studies
3—teaching
5—no information

125—Total

It is anticipated that many of those currently in military service will return to the dairy industry field. Those still in school are studying for either baccalaureate or advanced degrees. Eight of these are working towards a master's degree, three for doctorates, two for veterinary degrees, and one for the M.D. degree.

In concluding his report, Mr. Young states . . . "There is every indication that encouragement and recognition at the college level has proven of value in the results shown by the men and women to whom the award has been made. The educators making the nominations have selected an outstanding group who are continuing in their chosen field. It seems assured they will number many future leaders in education, production, and plant management in this important industry."

OUR ASSOCIATION

Dollars and Sense—and Nomination Time

DEAR FELLOW MEMBERS:

Our Association, like other science groups of similar size and character, has found itself on an "escalator" in recent years leading to higher and higher operating costs. Between 1951 and 1953, our operating expenses increased from approximately $35,000 to $46,500, a change of about 33%. The Association's budget was $46,000 for 1954, $48,000 for 1955, and is $51,000 for 1956.

The basic need of these various educational and science groups is to provide for adequate financing in the face of rather marked increases in expenditures. In our Association, the rise in operating expenses has not been offset consistently by proportionate increases in income from memberships, subscriptions, and advertising. Our Association operated at a deficit in 1953 and 1954 to the extent of about $4,900 and $6,400, respectively. The final financial statement for 1955 is not yet available, but it is anticipated that a more favorable income picture will be revealed. Whether this will be offset by the additional expenses related to the 50th Jubilee Year is yet to be determined.

Increases in operating costs of organizations such as ours have resulted largely from changes in printing costs for the official publications. The costs for the Journal rose $7,500 between 1952 and 1954, yet increases in income from memberships and subscriptions amounted to only about $3,300, an unfavorable balance of $4,200. Also, advertising revenues decreased about $2,000 between 1952 and 1954.

On the basis of the above figures, it is logical for the members to ask "What has been done to stop this unfavorable trend and to insure a balanced budget?" The answer is that definite steps were taken to correct this situation by the Executive Board in its June meeting in 1954. Membership and subscription rates were increased to bring them more nearly in line with those of comparable organizations. Reprints of publications were, for the first time, more logically priced, so that the agency supporting the research bore its share of the printing costs. Advertising rates were raised to a more reasonable level, and a positive program was initiated to increase the income from this source. Definite action was taken to increase the number of members and subscribers. The results of these steps will be awaited with interest when the financial report is presented at the June meeting in Connecticut.

But what about the future? Are we to have adequate financing for necessary growth and for the introduction of other needed changes?

First of all, we must realize that the administrative costs of our Association have been mounting and may be expected to become greater as time proceeds. To date, both the editor's and secretary-treasurer's positions are on a part-time basis. However, we have been fortunate in having men in these positions who give a great deal more time, effort, and interest than is called for by the budgetary arrangements. The questions are, "How long can we expect the Association to continue on this basis? How long can we expect to find persons to fill the editor's and secretary-treasurer's positions who will give so much for so little?" Obviously, the answer lies in a realistic view of this entire matter of Association administration and the acceptance of the fact that increases in these costs are inevitable in the not-too-distant future. Additional help for these key persons may be one of the answers.

In this connection, too, it should be recognized that the administrative load of the Association is increasing yearly, which requires more and more work in the offices of the secretary-treasurer, the editor, and the president. Some means must be achieved of lightening or distributing these administrative duties and financing adequately the related costs.

Improvement in our Journal cannot cease. The Journal Management Committee and Editor Tracy are constantly considering new ways to make the Journal more readable, to secure more advertising, and to increase sales appeal. They are aware that the main purpose of the publication is to disseminate the latest in scientific information. At the same time, they realize that the acceptance of the Journal by a large number of qualified readers who are not necessarily scientists demands a high degree of readability and the incorporation of material of general interest. Continued changes may be expected, and these improvements will cost money.

Because of the many major issues that arise as a part of the growing pains which the Association is experiencing, it is becoming increasingly necessary that the officers give additional time to Association affairs by meeting in formal session. At the present time, only one Board meeting is held each year and that in conjunction with the annual meeting of the Association. Even this meeting the Board members attend usually at personal expense, since the Association's budget does not provide for major coverage of these related costs. For several years, an off-meeting session of the Board has been suggested, probably for December or January. Such a meeting would require the financial support of the Association.

As our Association expands in its cooperative programs with other organizations, it is obligated to pay its proportion of the costs and to provide expenses for designated representatives on the inter-organization committees. These costs will need to be included in the Association's budget.

These specific examples illustrate the fact that the Association's costs are going UP. Consequently, we shall need to do a great amount of careful planning, make some positive de-

cisions, and take definite action if we are to meet these financial demands.

To some, the first obvious approach to a solution of this financial problem would be to improve the efficiency and reduce the present operating costs. This offers little promise. The operating budget for 1956 is pitiably low for the job which must be done, and any saving that may be effected by "cutting back" will be reflected immediately in a backward step in the progress of the Association. The present hope lies in increasing the revenue from the four sources—membership, subscriptions, advertising, and reprints.

Of these sources of income, those of memberships and subscriptions offer excellent possibilities. Since so much has been said in the Journal relative to this year's membership and subscription campaign and the individual member's responsibility in it, it would appear repetitious and superfluous to again stress this point. Nevertheless, I shall do so since we find ourselves faced with this dilemma: Either we increase the number of members and subscribers appreciably or look forward to paying more ourselves in annual dues and subscriptions. It is rather startling to realize that the Association had about 1,750 members and 1,500 subscribers during 1955—a ridiculously low figure when we consider the number of qualified persons and companies in our industry. Also, it is important to note that about 60% of the subscribers are in foreign countries. Increasing both memberships and subscribers by 250 during early 1956 would appear to be a small goal indeed, but this would help greatly to create a healthier financial situation.

As has been indicated in previous issues of the Journal, we can help as members by emphasizing the fact that all professional dairymen should become affiliated with our group. The first months of a year are excellent for bringing new members into the fold. It appears more and more important to tell the story of our Association and the role it plays in the dairy industry on every occasion possible. This can be done, not in a flamboyant, glamorous fashion but in a dignified manner befitting the stature of the organization. Since it is the professional organization of our industry and the industry's scientific and educational arm, our Association can point with pride to its accomplishments through its members in the fields of education, research, and industry. We need not be hesitant in promoting the Association on these bases.

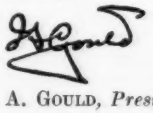
Time to nominate your officers. There is general acceptance in our Association of the principles of democratic action, and one of our greater privileges, as members, is to nominate and elect the officers. Your governing body, the Executive Board, is comprised of seven directors, the vice-president, president, secretary-treasurer (ex officio), and Journal editor (ex officio). Each year two directors and the vice-president are nominated and elected by the membership at large. The Nominating Committee, of which E. O. Herreid of the University of Illinois is chairman, now await your nominations for the positions to be filled at the Connecticut meeting. These positions include two directors (one from the Extension and one from the Manufacturing Section) and the vice-president (from the manufacturing interests). This is YOUR opportunity to have YOUR say regarding the new officers of the Association.

In respect to the Executive Board, the point is made now and then that the three sections (Extension, Production, and Manufacturing) do not have an equal number of representatives at all times. This is true, and this temporary, uneven distribution of Board members can be prevented only by a change in our constitution to enlarge the Board—a change which, to this date, has not been deemed desirable. However, it may be said emphatically that this slightly uneven distribution of section representatives need cause no concern to the members. In reality, sections are groups arbitrarily established on the basis of the subject matter of greatest interest, but there is no clear-cut line of differences between these groups from the standpoint of the affairs of the Association or the dairy industry as a whole. A director or an officer—even though selected from one subject matter area—is elected by the entire membership and is a representative of the whole Association. One needs only to visit the Board meetings and listen to the deliberations to realize that there is never any thought of differences due to sections, divisions, or groups—the decisions are based on "What is good for the Association."

Plans and committees. Plans are now well under way for the annual meeting in Connecticut. One important decision reached by the Program Committee is to have the Awards and Officers Installation program the last, rather than the second, night of the meeting. This is a change from the schedule followed in recent years. A Past Presidents' dinner will be held on June 18, and the charter members are to be honored at the opening ceremonies on June 19.

A committee has been appointed with representatives from the Eastern, Southern, and Western Divisions to consider how to bring about a closer relationship between the divisions and the parent organization. This committee probably will have recommendations to present before the meeting in June. The integration of the programs of these groups should help strengthen the internal relations of our Association.

Cordially,



I. A. GOULD, *President*

OUR INDUSTRY TODAY

Brief Reviews of Current Topics

Milkstone—It Can Be Controlled

J. G. LEEDER

Department of Dairy Industry
Rutgers University, New Brunswick, N. J.

Milkstone is a complex heterogeneous mixture of organic and inorganic substances which adhere tenaciously to milk heating surfaces. It is a substance closely related to boiler scale in steam generators, the relationship being that both are the result of heat treatment. The hard-water minerals cause boiler scale in a manner similar to the formation of milkstone by the mineral salts of milk.

Temperature a Factor in Formation of Milkstone

The amount of milkstone formed in a piece of milk-heating equipment, as well as its composition, bears a direct relationship to temperature. It has been shown by chemical analysis that the milkstone formed at low temperatures (vat pasteurization) is largely composed of adhesive fat and protein material. However, at higher temperatures (short-time, high-temperature pasteurization, 161° F. and higher) large amounts of ash materials, especially calcium and phosphorus, are precipitated. It has been further shown that milkstone formation is greatest at the temperatures that are favorable to the coagulation of albumin. X-ray studies have confirmed the composition of milkstone films.

There is some confusion about the terms milkstone and burn-on. Burn-on is the material "cooked" onto the surface of milk-heating tubes, plates, or jackets, and it consists largely of the solids found in milk. Alkaline detergents will easily remove the burn-on material from these surfaces. Milkstone, however, being largely composed of milk minerals from the ash portion of milk, is not readily removed by alkaline detergents alone but must be subjected to acid treatment for complete removal.

Many factors, in addition to temperature, influence milkstone formation. Among these are rate of heating, rate of flow of milk through equipment, type of product processed, amount of product processed, type and construction of equipment, kind of metal involved, and the surface area used for heating.

The formation of milkstone is the cause of one of the most important problems with which the milk plant operator must cope, namely, the cleaning of heating equipment. The use of HTST pasteurization equipment, as well as high-temperature preheaters and forewarmers, has greatly increased the milkstone problem because in these units large volumes of milk are heated to very high temperatures in relatively confined spaces.

FIG. 1. Photograph of milkstone deposit in a sanitary milk line.

Figure 1 shows a deposit of milkstone in a sanitary milk line used to carry hot milk from a heater. Milkstone usually does not form in a line where there is no heating taking place; there may be a slight deposit, however, in such lines, which will gradually become heavier if the film is not removed every day. Equipment with a heavy layer of milkstone can be discovered by merely looking at the surface when it is dry, but light films may go undetected for

a long time unless special examination is made. Ultra-violet light causes milkstone deposits to fluoresce in the dark, and special lamps are now available for detecting milkstone in pipes and vats and in other difficult and remote places.

The removal of milkstone is a must with the dairyman. It is the most common source of high thermoduric counts in pasteurized milk, since these organisms are afforded excellent protection when imbedded in the milkstone deposit. These bacteria can be the cause of poor keeping quality, off-flavors, and objectionable odors in dairy products.

Milkstone acts as an insulator in milk-heating equipment, and if not removed regularly it will reduce the rate of heat transfer and consequently the heating capacity of the equipment.

Prevention Methods

What can the dairy plant operator do to prevent the formation of milkstone in his milk product–heating equipment? It has been found that high velocity flow through small tubes will inhibit the formation of milkstone to a certain extent. However, even equipment which has the most favorable flow conditions will become inefficient in a few hours if cold milk is heated directly to temperatures at or above the boiling point of milk. By heating the milk in two stages, say, up to 190° or 200° F. in the first-stage preheater and then up to final temperature in a second-stage heater, the milkstone deposit can be greatly reduced. If the milk-heating equipment is designed so that there is a holding tube, surge tank, or hotwell inserted in the system between the two stages of heating, a large amount of the albumin precipitate can be eliminated from the high temperature tubes of the second-stage heater. Equipment employing these principles has been designed and operated with final temperatures as high as 280° F.

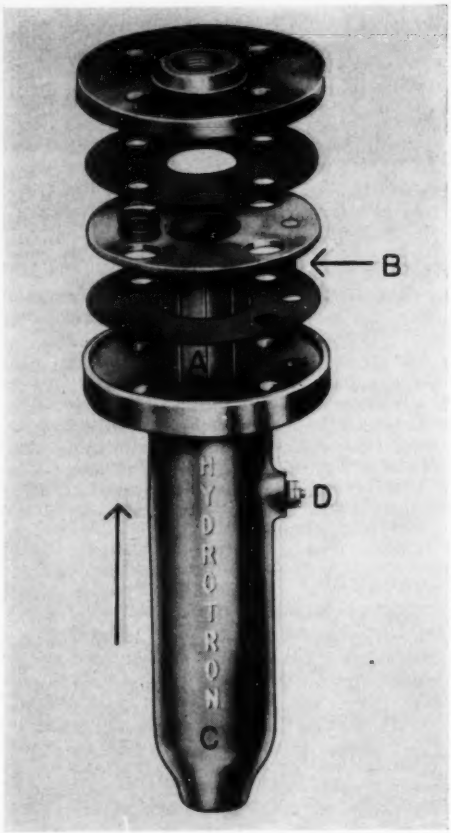


FIG. 2. An exploded view of the Hydrotron; A = inner tube, B = positive pole of circuit, C = housing of unit, D = negative pole of circuit.

TABLE 1

Results of commercial installation of the Hydrotron on milk precipitation in a short-time pasteurizer

Trial	No. of gal. treated	Milli-amperes	Cleaning time *(min.)*		After circulation[a]
			Acid	Alkali	
1a	2,970	Control	25	25	Plates clean
1b	3,970	1.0	10	20	Plates clean
2a	2,940	Control	10 + 17	20	After 10 min. of acid, precipitate still very hard and held to plates; 17 min. more of acid circulation needed
2b	3,450	1.0	10	15	Plates clean
3a	3,910	Control	10	10	Plates of regenerative section clean; heater section had gritty material in large amounts; brushing necessary
3b	4,000	1.0	10	10	Plates clean

[a] Before circulation, milkstone precipitate to 18th hot plate of regenerator, protein-like (sticky); all heater plates were gritty with mineral precipitate.

A unit called the Hydrotron has been used experimentally on a commercial HTST milk pasteurizer. It is an instrument developed and patented by A. G. Freeborn, an English engineer. (See Figure 2.) It prevents boiler scale formation in all types of steam generators. The instrument uses a small electrical current to charge a conducting cell through which the water passes, and this treatment affects the crystalline structure of the minerals when they are precipitated.

This unit was placed in the raw milk line of the pasteurizer so that all the milk entering the pasteurizer would be treated with the small electric current provided between the electrodes of this device. Tests made with this experimental installation indicated that the use of small electric currents in milk has an effect upon the amount and character of the milkstone which is formed by high-temperature, short-time heating. The important factors involved were found to be the rapidity of heating, the final temperature attained, and the current application from the Hydrotron. The optimum setting of the Hydrotron for the prevention of milkstone formation was in the range of 1.0 to 2.0 milliamperes.

The data in Table 1 show that with the Hydrotron in operation, the normal acid and alkali circulation times required to clean the plates could be reduced by more than one-half and it was not necessary to brush the plates after circulation when the Hydrotron was used.

What effect the Hydrotron would have in heaters operated above the boiling point of milk is not known, but indications are that the milkstone film would be reduced, if not entirely prevented.

Removal of Milkstone

It is not possible to completely prevent the formation of milkstone in high-temperature milk-heating equipment despite the use of various procedures or types of heaters described above. It is important therefore to know how to remove the milkstone film from heating surfaces. Ordinary alkaline cleaning compounds are ineffective when used alone. It is necessary to employ special organic acids, as well as alkaline cleaners. For this type of cleaning a recirculating system must be arranged so that the acid solution and the alkaline solution can be forced through the heater for 20 to 45 minutes. With alternate cleaning, the acid is circulated first and drained out of the system, and then the alkali is circulated. Recently one of the manufacturers of cleaners has developed a system for circulation cleaning of heating equipment in which the alkali solution is added directly to the acid after the acid has circulated for a while. By this means the salts of the organic acids are produced, which have definite chelating or sequestering properties that are advantageous in hard-water areas. Sequestering the hard-water components prevents their precipitation as water films on equipment surfaces.

During the last 4 or 5 years the C.I.P. (cleaned-in-place) system of cleaning sanitary pipe lines has become a widely adopted and successful operation. Milkstone has not been a problem in pipelines cleaned in this manner since no heating takes place in them. Where only hard water is available for flushing and cleaning C.I.P. lines, waterstone deposits may be a problem. By using soft water or by using alkaline detergents containing sufficient sequestering ingredients, waterstone may be controlled; however, if waters with a hardness of 25 grains or more per gallon are used, an organic acid detergent may be required at least every second day.

By using well designed equipment and following the procedures for heating milk to high temperatures as outlined, the dairy product manufacturer should not have too much trouble with milkstone. By proper circulation-cleaning of equipment with the correct cleaning agents, a small amount of milkstone can be easily and efficiently removed.

The Selection and Use of Nonfat Dry Milk Solids in the Manufacture of Cottage Cheese

RANDALL WHITAKER

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The employment of dry nonfat solids in the manufacture of cottage cheese has increased steadily since World War II. This has been due chiefly to the following factors:

1. The scarcity of fluid skimmilk at certain times.
2. The introduction of low heat spray powder after the importance of the heat treatment was fully recognized.
3. The discovery that the cottage cheese making operation is improved and the yield per pound of solids is higher when the total solids of the milk for setting is increased.
4. The recognition that more cottage cheese may be produced per vat when standardization of the total solids is practiced.

Dry nonfat solids may be used to advantage to raise the total solids of liquid skimmilk and

also to provide a suitable source of milk at times when fresh skimmilk is unavailable or priced excessively high.

Selection of Dry Nonfat Solids for Cottage Cheese

When skimmilk that has been pasteurized at higher than normal temperatures is used in the manufacture of cottage cheese, the cheese maker experiences difficulties because of the weak character of the curd. Curd weakness is objectionable during cutting because a uniform cut is difficult to obtain. Curd weakness is objectionable during cooking, as the particles shatter readily and curd losses in the whey are excessive. Whey expulsion from weak curd is slower, a longer cooking time is required, and the curd particle size is smaller as a result of greater shrinkage. Reconstituted skimmilk may be expected to present more difficulties than fresh skimmilk because of the heat treatment accorded the solids during the manufacture of the powder. Excessive pasteurization temperatures and heat treatment of the milk in the drier should be avoided in the manufacture of dry skimmilk for cheese making. The great importance of proper heat treatment dictates that only spray-process dried skimmilk may be used for the manufacture of cottage cheese. Roller type powder is unacceptable.

Various means have been suggested for appraising low heat powder, as it has come to be called in the trade, for use in cottage cheese. No one test performed on the powder has been developed that will permit reliable appraisal of dry milk solids for cottage cheese. Of the several tests that have been suggested, the determination of whey protein nitrogen (WPN) value is probably the most widely used means of estimating the suitability of powder for cheese making. This is a measure of the amount of protein nitrogen found in the filtrate from the coagulum formed from the reconstituted sample under carefully controlled conditions. The more heat that is applied to milk, the more the albumin is coagulated, and, since the protein in whey is chiefly albumin, it follows that low heat powders have a higher WPN value than high heat samples. A WPN value of at least 7.0 mg. is generally considered desirable for powder for cottage cheese. Natural variations in the whey protein content of milk limit the exactness of this test.

Curd tension is another factor that is somewhat indicative of the extent to which milk has been heated. Heat lowers the curd tension; hence a high curd tension is desirable in a low heat powder.

Miniature cheese making tests are valuable on occasion. Small vats, holding 1 or 2 qt. of reconstituted milk, are set under standard controlled conditions. On coagulation, the curd is cut with miniature curd knives and cooked under time and temperature conditions as similar to commercial practice as possible. Visual observations are made on the curd and whey, and it is possible, with some experience, to appraise powders for their cheese making properties fairly accurately.

One or more of these tests, coupled with the usual Dry Milk Institute analysis for flavor, solubility index, etc., will give an indication whether a given sample of dry skimmilk will be acceptable for making cottage cheese. Until a dependable test is devised or the present tests are made more reliable, the industry is relying on the integrity of reputable manufacturers to produce a uniform supply of low-heat powder. The quality is controlled by careful uniform processing and a testing program using one or more of the three tests mentioned.

The Use of Dry Nonfat Milk Solids in Cottage Cheese

Reconstitution. Several methods of reconstituting dry milk are employed by cottage cheese manufacturers. The most popular one consists of a centrifugal pump which draws water or skimmilk, if it is used, from a vat and feeds it back into the vat again, preferably as far away from the outlet as is practicable. The powder is introduced through a tee in the pipe line just before the pump. The suction created by the liquid flowing to the pump pulls the powder into the line and thence to the pump, where the violent agitation disperses the powder. The powder can be introduced into the tee from a hopper, or it may be sucked through a flexible tube directly from the bag or barrel. Unless care is exercised, excessive foam is formed and, in some cases, this limitation is so great that the reconstituted milk must be heated to destroy the foam.

Another method of incorporation is by means of a high speed mixer mounted on the side of the vat. In this system, the powder is generally shaken on the surface of the water or skimmilk in the vicinity of the vortex caused by the mixer.

A mechanical mixer, originally designed for dispersing chocolate powder for chocolate milk, is utilized in some plants. This hangs on the edge of the vat and consists of a rotating blade just below the surface. The powder is fed to the blades from a hopper. The blades are so designed that there is a strong downward flow of the liquid, which gives a rolling motion to the contents of the entire vat.

In some instances the dry milk is merely sprinkled on the surface of the skimmilk or water in a pasteurizer. This is slow and is not always sufficiently effective to eliminate all undissolved particles of powder.

Standardization. Standardization to a total solids content of 9 to 12% is generally practiced. Lower concentrations are sometimes employed when fresh skimmilk is fortified with

powder, whereas the higher amounts are preferred when all reconstituted skimmilk is used. Milks containing up to 20% total solids have been used, but best results are obtained at the 9-12% level. Standardization of the solids of skimmilk to this level is desirable because:

1. The curd is firmer. This helps the cheese maker during cutting and cooking, and the curd will withstand more abuse. It can be agitated more thoroughly, thus promoting uniformity.

2. Higher pasteurization temperature may be employed. Standardization counteracts the weakness of curd that results from heating milk.

3. Less experienced help may be used. Handling a weak curd requires experience, whereas with the firmer curd obtained through standardization less experienced help is needed. Reconstituted milk is more uniform than fresh skimmilk, and daily variations are less pronounced.

4. It permits the use of some homogenized milk. The weakness of curd that is noted when homogenized fat is present may be counteracted in part by increasing the total solids through standardization.

5. The yield is higher. More pounds of curd are obtained per pound of nonfat solids in the milk as the total solids of skimmilk are increased.

6. The cheese vat capacity is increased.

7. It shortens the cheese making process. The firmer curd characteristics permit faster heating. The final heating temperature is generally lower when standardized milk is used.

Pasteurization. Pasteurization of reconstituted skimmilk is the exception rather than the rule unless local regulations require it. The heat treatment given the milk during the manufacture of dry nonfat solids, although held to a minimum, is sufficient to reduce the microorganism content to a low level, and pasteurization of the reconstituted milk is unnecessary. Properly pasteurized fresh skimmilk and a good water supply are, of course, essential in the manufacture of quality cottage cheese. Where pasteurization is required, the curd weakness resulting from the extra heat is restored through standardization of the total solids of the milk.

Setting. Reconstituted milk is set in the same manner as fresh skimmilk, using either the short- or long-set methods, a good active starter, and a prescribed amount of a coagulator or rennet.

Cutting. Since most cheese makers using dried nonfat solids practice standardization, the cutting acidity is selected on the basis of the total solids in the milk. The whey acidities most commonly used are as follows:

% total solids in skim	*% whey acidity at cutting*
8	0.48-0.50
9	0.50-0.52
10	0.52-0.54
11	0.54-0.56
12	0.56-0.58

The size of the curd knives determines in large measure the size of the curd particles and is governed by the type of product desired. Curd from milk standardized to 11-12% solids shrinks slightly less than curd from fresh skimmilk; hence ½-in. knives are generally used when ⅝-in. knives give the desired particle size with fresh skimmilk. Fine curd cottage cheese made from reconstituted milk is usually made with ¼-in. curd knives.

Cooking. The cooking process is the same as for fresh skimmilk. When the milk has been standardized to 11-12%, the final cooking temperature is usually slightly lower than when fresh skimmilk or low total solids reconstituted milk is used. The final cooking temperature determines the curd firmness and, of course, is varied by the cheese maker to suit local demands. Greater uniformity in cooking is generally obtained with standardized reconstituted milk than with fresh skimmilk. This is especially true when the dry nonfat solids are obtained from a single reliable manufacturer who markets a brand of low heat powder adequately backed by a good quality control program.

Washing, draining and creaming. Curd made from reconstituted milk behaves just the same as curd from fresh skimmilk, and no special precautions are required in handling it at this time.

Future Use of Dry Nonfat Solids in Cottage Cheese

It seems logical to believe that the use of nonfat solids in the manufacture of cottage cheese will expand. The appreciation of the advantages of using powder is growing. Shortages in the supply of fresh skimmilk at a reasonable price seem to be increasing, especially in the South. The year-by-year increase in the annual production of cottage cheese and a greater annual consumption resulting from better advertising of the nutritive value of cottage cheese should contribute to greater utilization of dry nonfat solids in cottage cheese in the years ahead.

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ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL DISEASES

132. An outbreak of bovine mastitis associated with dry storage of teat cup inflations. J. SIMON and R. HALL, Animal Disease Diagnostic Lab., Madison, Wisconsin. J. Milk and Food Technol., **18**, 12: 299. 1955.

The increased frequency of mycotic mastitis may be the result of the antibiotic age. Dry storage of teat cup inflations may contribute to the spreading of bovine mycotic mastitis. *Cryptococcus neoformans* and *Candida* sp. have been isolated from bovine mastitis. The organisms are indistinguishable from pathogenic human strains. H. H. Weiser

BOOK REVIEWS

133. Farm Service Buildings. HAROLD E. GRAY. McGraw-Hill Book Company, Inc., New York, N. Y. 458 pp. $7.50. 1955.

While the book is written to serve as a text for a college level course in farm structures, it should prove a helpful guide and source of information to dairymen and others interested in the subject. The newer developments in farm building, planning, and construction are presented along with standard methods.

The book is well-organized and well-written. It consists of seven parts, each of which in turn is divided into chapters which cover a specific subject. The parts are: Development and Planning of Farm Buildings; Materials of Construction; Structural Requirements; Environmental Control; Buildings for Housing Livestock; Storage Buildings; Remodeling.

Drawings and pictures are freely used throughout the book. The text is well-documented with reference to source materials. The index is complete and unusually usable.

The basic principles expressed in parts one through four are applicable to the problems of dairy cattle housing and other buildings associated with dairy farms. Chapters 16, Dairy Cattle Housing; 23, Silos; and 24, Remodeling Farm Buildings are particularly designed to serve the needs of those interested in farm buildings pertinent to the dairy enterprise.

E. E. Ormiston

CHEESE

134. New curd meter takes guesswork out of cheesemaking. E. LUNDSTEDT, H. P. Hood and Sons, Boston. Food Eng., **27**, 11: 97. 1955.

The Hood Curd Meter is a recently developed instrument for measuring the compressibility of cottage cheese curd during manufacturing. This measurement is related to the texture and firmness of the curd, and as such is a guide to correct cooking treatment. Use of the meter eliminates the variability arising from compressing the curd by hand to determine the correct firmness. The meter is a plunger type and has a graduated scale, reading from 0 to 26. Normal uncreamed curd in the New England area gives a reading of 6. In cheesemaking, drainage of whey at a curd reading of 10 results in a suitable final curd. Data presented from various experiments using the curd meter, indicate that each vat of cheese has a critical temperature of cooking. Even experienced cheese makers were not always able to determine the proper cooking treatment required. Using the curd meter and a slow uniform cooking procedure, it was possible to obtain uniformity of curd texture and a final meter reading of 6. Conditions need to be altered when part whole milk is used or when utilizing low heat powders. T. J. Claydon

135. Dip coating puts cheese wedges back up front. Food Eng. Staff, Food Eng., **27**, 11: 70. 1955.

Tillamook County Creamery Assn., Ore., has developed a mechanized system for dip coating cheese wedges. A cutting head with stainless steel wires cuts the wedge-shaped slices. Weighed wedges are impaled on prongs and conveyed through the coating machine. The process involves dipping in thermo-plastic wax at 150° F. and then in plastic. After passage through a drying chamber the dipping process is repeated. Following final drying, wedges are labeled, marked with the exact weight, and cased. Present production is 3,000 12-oz. units per 8-hr. day. The system makes wedge packaging economically feasible, provides the best available protection for the cheese, and eliminates labor, cutting, and moisture losses at the retail point.

T. J. Claydon

DAIRY BACTERIOLOLGY

136. Report on fungicide and germicidal rinse testing methods. L. F. ORTENZIO, U. S. D. A., Washington, D. C. J. Assoc. Offic. Agr. Chemists, **38**, 2: 274. 1955.

A procedure is outlined in the book "Antiseptics, Disinfectants, Fungicides, and Sterilization" by L. S. Stuart, for determining the available chlorine germicidal equivalent concentrations of disinfecting solutions. In the tests reported, the method as outlined by Stuart was used except that the pH 8.0 phosphate buffer was supplanted by a pH 8.5 buffer and the test culture increment volume of 0.2 ml. was changed to 0.05 ml. Experiments with the method showed that 200 p.p.m. available chlorine as sodium hypochlorite was equivalent to 25 p.p.m. of titratable iodine. However, with solutions containing 12.5 and 6.25 p.p.m. of titratable iodine, the results were not quite as good as results obtained with solutions containing 100 and 50 p.p.m. of available chlorine, respectively. F. J. Babel

137. The phenol coefficient number as an index to the practical use-dilution for disinfection. L. S. Stuart, L. F. Ortenzio, and J. L. Friedl, U. S. D. A., Washington, D. C. J. Assoc. Offic. Agr. Chemists, **38**, 2: 465. 1955.

The phenol coefficient procedure for determining activity of germicides was criticized because the activity of germicides does not increase directly as the concentration increases. Data from 125 commercial and laboratory compounded preparations indicated quite clearly that neither the constant conversion factor of 20 now commonly employed, nor a conversion formula based on a simple exponential relationship between concentration and germicidal activity, could be relied upon to convert phenol coefficient numbers to safe dilutions for use in the absence of other information. The results clearly pointed out the necessity of employing analytical chemical data and/or concentration exponent data in making estimates of the dilutions suitable for actual use in disinfecting.
F. J. Babel

138. Report on sporicidal tests for disinfectants. J. L. Friedl, U. S. D. A., Washington, D. C. J. Assoc. Offic. Agr. Chemists, **38**, 2: 280. 1955.

A sporicidal test is outlined which is applicable for use with germicides to determine the presence or absence of sporicidal activity and potential effectiveness in disinfecting against specific spore-forming bacteria. Data are presented to illustrate the danger of drawing conclusions relative to the sterilizing activity from the results obtained with resistant spores of a single species. One germicide killed spores of *Bacillus subtilis* in 10 to 30 min. while it did not kill spores of *Clostridium sporogenes* in 2 hr. F. J. Babel

139. The sporulation of Bacillus sphaericus stimulated by association with other bacteria: An effect of carbon-dioxide. J. F. Powell and J. R. Hunter, Microbiol. Research Dept., Porton, Wiltshire, England. J. Gen. Microbiol., **13**, 1: 54. 1955.

Sporulation of *Bacillus sphaericus* was found to be stimulated by growth of these cultures in association with several other spore-forming organisms, as well as a number of other gram-positive and gram-negative bacteria. Sporulation in pure culture was induced by the presence of bicarbonate and ketoglutarate, and the conclusion was reached that carbon dioxide was the stimulating factor in mixed cultures.
J. J. Jezeski

140. Spore germination in the genus Bacillus: The modification of germination requirements as a result of preheating. J. F. Powell and J. R. Hunter, Microbiol. Research Dept., Porton, Wiltshire, England. J. Gen. Microbiol., **13**, 1: 59. 1955.

Germination studies were conducted on spore suspensions of *Bacillus megatherium*, *Bacillus cereus*, and *Bacillus anthracis*. Spontaneous germination was observed in spore suspensions of *B. megatherium* after heat treatment; this germination was not inhibited by heating with cyanide, azide, 2,4-dinitrophenol, or sodium iodoacetate. Spores of *B. cereus* had a germination requirement of either inosine or a mixture of alanine, tyrosine, and adenosine; but after heat treatment, only adenosine alone. *B. anthracis* spores after preheating required either inosine or adenosine, tyrosine, and alanine. J. J. Jezeski

141. Correlation between morphological and physiological characters in the classification of members of the genus Lactobacillus. G. H. G. Davis, K. A. Bisset, and C. M. F. Hale, Dept. of Bacteriol., Univ. of Birmingham, England. J. Gen. Microbiol., **13**, 1: 68. 1955.

The morphological and cytological characteristics of 221 strains of the genus *Lactobacillus* were studied. The cultures included 113 heterofermentative and 108 homofermentative strains. Marked morphological differences were observed between homofermentative and heterofermentative strains as well as between physiological groups within the homofermentative group. J. J. Jezeski

142. Washing and sanitizing the cow's udder. A. V. Moore, Texas Agr. Expt. Sta., College Station. Milk and Food Technol., **18**, 12: 314. 1955.

Bacteria counts of fresh milk are lower if a large volume of plain water is used to rinse the udder, as compared to wiping the udder only with a hypochlorite-soaked cloth. The best results are obtained when a water rinse precedes wiping with a bactericidal solution. A cloth soaked in 200 p.p.m. hypochlorite is more effective than one soaked in the same strength quaternary ammonium solution.
H. H. Weiser

143. The effect of freezing on the standard plate count of milk. C. K. Johns and I. Berzins, Can. Dept. of Agr., Ottawa. J. Milk and Food Technol., **18**, 12: 297. 1955.

The microflora of milk and cream samples was subjected to quick freezing at –17.8° C. Similarly, samples were exposed to slower freezing at –10° C. The bacterial count was less than the count for liquid whole egg at –19° C.

The authors assume that there are more Gram positive bacteria in milk and cream and they are more resistant to freezing. While in liquid egg Gram negative bacteria are predominant. H. H. Weiser

144. Use of microorganisms as analytical tools. D. A. HARRIS, Research Labs., Chem. Div., Merck & Co., Inc., Rahway, N. J. Analyt. Chem., **27**, 11: 1690. 1955.

Microorganisms can be used successfully to assay various antibiotics, vitamins, and amino acids with an acceptable degree of accuracy even when crude and unknown types of materials are involved. In general, they are inexpensive to run, are not difficult to handle, and can be adapted for large-scale operations. The characteristics of the bacterial growth curve are discussed. Serial dilution, diffusion, and turbidimetric methods are described. Interfering substances sometimes cause assay drifts during the vitamin and amino acid assays.

In many cases microorganisms represent the only satisfactory analytical tool available for use in specific chemical, stability, biological standardization, nutritional, and microbiological studies. B. H. Webb

DAIRY CHEMISTRY

145. Report on detection of reconstituted milk in fluid market milk. C. L. CLAY, M. B. BURKE, and G. JUNKER, La. Dept. of Health, New Orleans. J. Assoc. Offic. Agr. Chemists, **38**, 2: 310. 1955.

Improvements in manufacturing methods for non-fat dried milk solids have rendered the Evenson (J. Dairy Sci., **5**, 97. 1922) and related methods obsolete for detecting reconstituted milk in fluid milk. A new method is presented which is based on an increase in the protein-reducing substances of fluid milk when reconstituted milk is added thereto. With the method, addition of reconstituted milk in excess of 20% can be detected when the P.R.S. (protein-reducing substances) value of the raw milk is not known. When the P.R.S. value of the raw milk is known, the method detects as little as 5% of reconstituted milk.

F. J. Babel

146. Rapid method for water-insoluble acids (WIA) in butter. L. G. ENSMINGER, Food and Drug Admin., Cincinnati, Ohio. J. Assoc. Offic. Agr. Chemists, **38**, 1: 183. 1955.

A method is described in which butter is washed in a separatory funnel with hot water, the water separated from the fat, and the fat titrated with sodium ethylate in ether solution. A formula is given for converting the titration value to mg. WIA per 100 g. fat. Comparisons made with the official method and the rapid method on 11 samples of butter (39 determinations) showed that the av. for all determinations by the rapid method was 333.6. The greatest positive deviation by the rapid method was 57 mg. as compared to a max. negative deviation of –31 mg. Erratic results were obtained with the rapid method on cream because of the interference of proteins. F. J. Babel

147. Identification of fats by urea fractionation. W. F. SHIPE, Dairy Dept., Cornell Univ., Ithaca, N. Y. J. Assoc. Offic. Agr. Chemists, **38**, 1: 156. 1955.

Fractionation of fat by means of urea was used as a method of identifying fats in butter and ice cream. Selectivity of the fractionation procedure was demonstrated by measuring the refractive indices of the various fractions. Experiments indicated that the type and amount of fatty acids that could be precipitated by urea complex formation was dependent on the urea concentration. The method appeared to have possible value as a means of detecting adulteration of butterfat with other fats.

F. J. Babel

148. Report on extraneous materials in dairy products. D. B. SCOTT, Food and Drug Admin., Washington, D. C. J. Assoc. Offic. Agr. Chemists, **38**, 2: 259. 1955.

Methods are outlined to replace the present methods for determination of extraneous materials in butter, evaporated milk, condensed milk, sweet cream, sweet milk, dried milks, soft and semi-hard cheeses, sour cream, hard skimmilk cheese, and cheeses containing mold, plant tissue, and spices. A method in which cheese is digested with enzymes, and in which some of the proteins are partially solubilized and then a colloidal solution in hot acid-alcohol solution is obtained, was found entirely satisfactory for hard skimmilk cheese.

F. J. Babel

149. Report of quaternary ammonium compounds. J. B. WILSON, Food and Drug Admin., Washington, D. C. J. Assoc. Offic. Agr. Chemists, **38**, 3: 557. 1955.

The technique of identifying crystalline compounds by their refractive indices as determined by the immersion method with the polarizing microscope appeared to be suitable for identifying the various crystalline quaternary ammonium reineckates. From a qualitative standpoint, both the brom-phenol-blue method and the eosin-yellowish method gave positive results when 2 p.p.m. of quaternary ammonium compounds were present in milk. From a quantitative standpoint, recoveries were greater by the eosin-yellowish method when small quantities of quaternary were present, but with larger amounts of quaternary recoveries were the same by both methods. F. J. Babel

150. Report on foreign fats in dairy products. Sterol acetate test. J. H. CANNON, Food and Drug Admin., St. Louis, Mo. J. Assoc. Offic. Agr. Chemists, **38**, 2: 338. 1955.

Experiments with a carbon column method for purification of sterols indicated that it was not as dependable as the digitonin method. The addition of cholesterol to coconut oil samples just before the digitonin precipitation gave higher melting acetates than those obtained without such addition. The sterol-acetate test could detect as little as 10% of added vegetable fat if conducted by skilled personnel.

F. J. Babel

151. Report on foreign fats in dairy products. A critical review of the Reichert-Meissl and Polenske determinations. S. D. FINE, Food and Drug Admin., Denver, Colo. J. Assoc. Offic. Agr. Chemists, **38**, 2: 319. 1955.

A comprehensive history of the methods for the determination of Reichert-Meissl and Polenske numbers is presented. The effects of certain variables in the tests are discussed. A drawing is shown of an apparatus with all joints of ground glass. A comparison was made between results obtained with the new (all glass) and old apparatus. Also, the type of fat, method of heating, the anti-bumping agent used, time of distillation, and amount of glycerol-soda reagent used were studied for their effect on Reichert-Meissl and Polenske values.

F. J. Babel

152. A detergent test for the milk fat content of dairy products. I. Milk, cream, and ice cream. O. S. SAGER, G. P. SANDERS, G. H. NORMAN, and M. B. MIDDLETON, U. S. D. A., Washington, D. C. J. Assoc. Offic. Agr. Chemists, **38**, 4: 931. 1955.

The BDI detergent test for butterfat was modified to improve the methodology, accuracy, and appearance of the fat column. Values obtained with the modified test on milk and ice cream agreed closely with those obtained by the Babcock and Roese-Gottlieb results. The detergent test results on cream averaged about 0.75% higher than the Babcock results and 1% higher than the Roese-Gottlieb results. The curved bottom meniscus in the detergent test on cream accounted for the increase over that of the Babock test. The detergent method conducted in silicone-treated bottles gave results which averaged the same as for the Babcock test and 0.25% higher than the Roese-Gottlieb test.

F. J. Babel

153. Sample preparation in the bioassay for vitamin D. L. FRIEDMAN and G. M. SHUE, Food and Drug Admin., Washington, D. C. J. Assoc. Offic. Agr. Chemists, **38**, 1: 165. 1955.

An attempt was made to develop a method for the bioassay of vitamin D in milk which could be adapted later to other products. With evaporated milk, it was found that simple extraction procedures would not recover all of the vitamin D; a saponification procedure was necessary for quantitative recovery. A saponification procedure is outlined which overcomes the difficulties in assaying foods low in vitamin D potency.

F. J. Babel

154. Variation in fat constants of genuine Canadian milk fat. R. R. RIEL, Can. Dept. Agr., Ottawa. J. Assoc. Offic. Agr. Chemists, **38**, 2: 494. 1955.

The range of values obtained for Reichert-Meissl, Polenske, and refractive index determinations was appreciably narrower in Canadian milk fat than are those reported in the literature. The av. values and 95% fiducial limits were: Reichert-Meissl 27.8 (25.1-30.5); Polenske 2.0 (1.4-2.6); and refractive index 1.45419 (1.45326-1.45512). There were significant differences between months and between provinces.

F. J. Babel

155. A modified Cornell phosphatase test for the analysis of blue mold and aged cheeses. J. H. MAHON, C. ANGLIN, and R. A. CHAPMAN, Food and Drug Directorate, Ottawa, Canada. J. Assoc. Offic. Agr. Chemists, **38**, 2: 482. 1955.

The Cornell procedure for the determination of phosphatase was modified to improve the reproducibility, to increase the linear range of the phenol calibration line, and to remove some of the abnormalities encountered in the determination of phosphatase activity in blue mold and aged cheeses. All cheeses depressed the slope of the phenol calibration line. The effect was especially marked with blue mold and aged cheese and ranged from 10 to 88% of the theoretical value. There was a relationship between the depressing effect of cheese upon the slope of the phenol calibration line and the free amino acid content of the cheese. The use of a 1:6 dilution for all cheese samples increased the slope, the analytical range, and the linearity of the phenol calibration line. Employment of a single dilution factor ensured that all results were comparable.

F. J. Babel

156. New studies made of solids determination. W. R. DAVEY and S. PATTON, Penn. State Univ., University Park. Milk Plant Monthly, **44**, 12: 23. 1955.

Total solids determinations are essential for the standardization of dairy products and to satisfy quality demands and legal requirements. There is no completely satisfactory total solids test for fluid milk having all the requisites of reproducibility, speed, simplicity, and low initial and operational costs. Studies of refractive indices measurements of milk-glycerol and milk-detergent mixtures; rapid drying of multiple samples using the Brabender Semi-Automatic Moisture Tester; certain modifications of the

Chemical Oxidation Demand test commonly used on dairy wastes; hydrolysis of acetyl chloride in the presence of pyridine by water with the production of acidity; Karl Fischer reagent for a titrimetric determination of water; a colorimetric procedure; and a turbidity titration were therefore made to determine their possible application to milk. The most promising procedures involved the use of the refractometer with diluents, the Brabender Semi-Automatic Moisture Tester and the Chemical Oxidation Demand method. The reproducibility of the refractometric and oven-drying procedures was limited by the instruments used in the determinations. The other methods, while exhibiting poor correlation with Mojonnier tests in this survey, cannot be discredited entirely since an exhaustive study of each procedure was not made. C. J. Babcock

157. Foreign fat characteristics. J. B. MICKLE, M. LOWENSTEIN, and C. L. BURTON, Okla. A. & M. Coll., Stillwater. Ice Cream Field, **66**, 4: 124. 1955.

The characteristics of five lots of fat were determined before and after being processed into ice cream. Two butterfat samples from cream in Aug. and Oct., 2 hydrogenated vegetable fat samples and one hydrogenated beef fat sample were included in this study.

Very little difference was observed between the original fats and those same fats after being recovered from the frozen desserts by a modified ether extraction procedure. Indications are that some differences would be expected if the fat is extracted by one of the modified Babcock procedures. W. C. Cole

DAIRY ENGINEERING

158. Paints for dairy plant buildings and equipment. A. E. NIEDT, Steelcote Mfg. Co., St. Louis, Mo. Ice Cream Rev., **39**, 5: 38. 1955.

The correct procedures for preparing various surfaces for painting along with the proper types of paint and their application are discussed. An epoxy-resin paint of recent origin which reportedly will produce a finish that has 75% of the hardness of glass and yet does not get brittle, which offers superior resistance to brine, lactic acid, heat, butter fat and moisture is especially recommended for use on bottle washers, sterilizing equipment, homogenizers, conveyors, water tanks, etc. The paint will adhere to any clean wet or dry surface and will dry in 1½ to 2 hours. In a weathering test, equivalent to seven years' outside exposure, the paint showed very little dulling. It is estimated that this paint should give from five to ten years of satisfactory service under the severe conditions which prevail around dairy plants.
W. J. Caulfield

159. Inverting apparatus. H. M. KENDALL (assignor to Nordica A. Kendall). U. S. Patent 2,726,779. 4 claims. Dec. 13, 1955. Offic. Gaz. U. S. Pat. Office, **701**, 2: 341. 1955.

A mechanism for up-ending milk cans over a weigh or other vat. R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

160. Advertising budget for milk dealers. ANON. Milk Dealer, **45**, 3: 50. 1955.

A survey conducted by The Milk Dealer, by the use of a questionnaire, relative to how the advertising dollar should be used, showed that a milk dealer should spend 1.13% of his sales dollar for advertising. This figure was the average of all replies, with expenditures ranging from nothing to 2%. The survey also showed that 67% of the dealers set up an advertising budget, and 85% of these base it on gross sales, while 15% base it on net sales. Some 62% of the dairies with advertising budgets set up their appropriations on the basis of their last year's sales, 8% on their last month's sales; 23% on anticipated sales for the coming year; and 7% on a combination of last year's sales and anticipated sales for the coming year. The services of advertising agencies were used by 60% of the dairies replying. Methods of advertising are also given. C. J. Babcock

161. Employee education program. C. R. DEHAVEN, Penn. Assoc. of Milk Dealers. Milk Dealer, **45**, 3: 53. 1955.

A detailed consumer opinion survey conducted for the Penn. Assoc. of Milk Dealers in Pittsburgh, Erie, Sharon, Meadville, and Reading clearly indicated that the consumer, for the greater part, was either ignorant or oblivious to important factors relating to pricing of milk. This, at a time when the industry was being subjected to almost daily criticism, showed a deep necessity on the part of the dairy industry to do something about prevalent wide-spread misconceptions. It resulted in PAMD's Employee Education Program to cultivate public confidence in our dairy system; to create an intelligent understanding of its social and economic importance and to build better relations for the individual milk dealers who compose it. Results of the survey and details of the program are given.
C. J. Babcock

162. Rising costs can be curbed. T. D. LEATHERS. Milk Plant Monthly, **44**, 12: 16. 1955.

The increased costs of today's milk plant operations can be lowered if the cost of manufacturing and selling each item is known. A careful study of each operation and a comparison with other plants will help show where cuts can be made. Elimination of duplication facilities, preventive maintenance, consultation

with employees, and cost consciousness are necessary if a lower cost program is to be successful. C. J. Babcock

163. Milk in spotlight at vending show. T. D. Leathers. Milk Plant Monthly, **44**, 12: 27. 1955.

In 1955 milk in vending machines showed the most substantial increase over 1954 sales of any vendible product. Both indoor and outdoor vendor sales of milk were estimated to increase 75% in 1955 over vended milk sales in 1954. A total of 22,000 indoor milk vendors will sell an estimated $30,800,000 in milk this year, while 2,400 outdoor units sell about $8,400,000. Meanwhile, ice cream sales through vendors in 1955 are estimated to be 15% higher than last year with the 22,500 vendors selling about $23,000,000. C. J. Babcock

164. The importance of location. Staff Editors. Milk Plant Monthly, **44**, 12: 40. 1955.

A survey at Clemson College of five coin operated vending machines showed that milk sales through vendors in dormitories had little effect on canteen or cafeteria milk sales, and that the availability of soft drinks did not influence milk sales through the vendors. Chocolate milk was the biggest seller at the college. Sales were greatest where homogenized, chocolate, and buttermilk were all offered and were lowest when only one product was sold.

C. J. Babcock

FEEDS AND FEEDING

165. The outdoor rearing of calves on grass with special reference to growth rate and grazing behavior. J. H. B. Roy, K. W. G. Shillam, and J. Palmer. N.I.R.D., Univ. Reading, England. J. Dairy Research, **22**, 3: 252. 1955.

A number of Dairy Shorthorn bull calves which were born in the spring of 1953 and 1954 were fed outdoors with continuous access to pasture from birth. They were first given colostrum and then approximately 45 gal. of milk over 8 wk. after which time they were gradually weaned. Except for the milk and a small quantity of wheat straw and dried grass during a period of dry weather all of the nutrients were obtained by the calves from pasture grass. The gains the first month were subnormal but thereafter gains were considered normal and health was normal in respect to scouring and incidences of infections which caused a rise in rectal temperatures. Rearing calves on pasture from birth was done with a high degree of success.

Grazing behavior studies were made periodically during the study and considerable data on this phase of the work is included in the report.

J. D. Donker

HERD MANAGEMENT

166. Cattle feeding device. R. G. Ferris (assignor to Starline, Inc.). U. S. Patent 2,726,635. 8 claims. Dec. 13, 1955. Offic. Gaz. U. S. Pat Office, **701**, 2: 303. 1955.

An arrangement for delivering feed to cows standing in stalls facing an alley.

R. Whitaker

ICE CREAM

167. Cutting costs in the ice cream plant. R. G. McCutcheon, Maola Milk and Ice Cream Co., New Bern, N. C. Ice Cream Rev., **39**, 5: 36. 1955.

A weekly one hour training program combined with an employee profit sharing plan has proved effective in reducing costs of operation in the Maola Milk and Ice Cream Company. Accomplishments of the program cited by the author include a 30% saving in washing compounds and an increase in novelty production from 4,600 to 5,500 doz. per 8 hour day with the same number of people.

The weekly one hour training program is divided into two parts. The first 45 min. are used to train and educate the personnel in how to do a more efficient job and to present progress reports on cost reductions that have been made. The last 15 min. of the weekly session is allocated to a local minister who talks to the group. W. J. Caulfield

168. Effect of storage upon nitrogen distribution. J. B. Mickle and J. A. Meiser, Mich. State Univ., East Lansing. Ice Cream Field, **66**, 5: 54. 1955.

It was found that with ice cream stored for 12 weeks at 4 to 6° F. total nitrogen of the top of pint samples increased with storage. Other nitrogen values at the top of the package also increased during storage, but these fractions did not change in their relationship to each other or to the total. Nitrogen values for the middle of the samples did not vary materially.

Shrinkage of ice cream increased with length of storage. The authors found no evidence of shrinkage being due to concentration of various nitrogen fractions at the top surface of ice cream. W. C. Cole

169. Shrinkage as affected by milk protein variations. J. C. Trautman and T. A. Nickerson, Dept. of Dairy Ind., Univ. of Calif., Davis. Ice Cream Field, **66**, 4: 127. 1955.

Results of experiments are reported in which several variables are considered so far as their influence on ice cream shrinkage is concerned. Decreasing the globulin content of ice cream increased the tendency for ice cream shrinkage. Using pepsin in ice cream mixes under varying

conditions it was concluded that proteolysis that takes place in the mix after pasteurization is more likely to cause shrinkage than proteolysis to the same degree before pasteurization.
W. C. Cole

170. Selling stick confections through super markets. C. O. Lund, C. O. Lund and Assoc., Inc., Chicago, Ill. Ice Cream Rev., **39**, 5: 32. 1955.

The results of a market test in the Chicago area dealing with the sale of stick confections through grocery stores are reported. Factors which were found to be important in the merchandizing of stick confections through super markets are described.

The test indicated that super markets will sell properly packaged units of stick confections. The author predicts that the sale of stick confections through super markets will increase the sale of ice cream and related products by many million gal. annually.
W. J. Caulfield

171. How it's done in Denmark. G. H. Wilster, Ore. State Coll., Corvallis. Ice Cream Field, **66**, 4: 82. 1955.

The author reviews briefly some of the developments which have taken place in the ice cream industry of Denmark. W. C. Cole

MILK AND CREAM

172. Performance characteristics of various bulk milk tanks. S. A. Witzel and C. O. Cramer, Dept. Agr. Eng., Univ. of Wis., Madison. J. Milk and Food Technol., **18**, 12: 306. 1955.

Direct expansion tanks required less power than the ice bank bulk milk tanks. However, both types compared favorably with mechanical can coolers at same temperatures. Some ice bank tanks were underpowered while others had insufficient ice bank capacity to cool the milk to 40° F. within two hours after milking. One direct expansion tank using air cooling did not meet cooling requirements when operating in the 84 to 88° F. temperature range.
H. H. Weiser

173. Refrigerator type milk container and dispenser. F. E. Billock and T. L. Billock. U. S. Patent 2,726,795. 1 claim. Dec. 13, 1955. Offic. Gaz. U. S. Pat. Office, **701**, 2: 345. 1955.

A 1-gallon rectangular shaped container for storing milk in a home refrigerator. A petcock is provided for dispensing the milk as required.
R. Whitaker

MILK SECRETION

174. Intramammary pressure changes in the lactating cow. I. Changes during the milking process. W. G. Whittlestone, Ruakura Animal Research Sta., Dept. Agr., New Zealand.

Pressure changes within the teats of adjacent quarters, one of which was concurrently being milked, were recorded. The pressures were first recorded immediately after milk ejection was stimulated by washing the udder. The pressure in the quarter being milked gradually fell but could again be materially increased by restimulating the teats and udder to effect a second let-down of milk. The pressure in the unmilked quarter subsided but very slowly and slightly over a period of 20 min. and the pressure did not materially change when the second milk ejection response was elicited. The milk obtained from the quarter from which recordings were taken was normal in amount only after the second ejection response had been elicited due to the fact that the milking time had been greatly prolonged. J. D. Donker

PHYSIOLOGY AND ENDOCRINOLOGY

175. Studies of the secretion of milk of low fat content by cows on diets low in hay and high in concentrates. VI. The effect on the physical and biochemical process of the reticulo-rumen. C. C. Balch, D. A. Balch, S. Bartlett, M. P. Bartrum, V. W. Johnson, S. J. Rowland, and J. Turner, N.I.R.D., Univ. Reading, England. J. Dairy Research, **22**, 3: 270. 1955.

Two fistulated Milking Shorthorn cows were subjected to experimental observation while on 5 different rations over a time of 27 wk. Data were recorded on milk production, fat content of milk, blood glucose and partition analysis of lipids of blood, analysis of rumen contents for volatile fatty acid and pH and the digestion which took place in the rumen alone and in total. The first ration consisted of 16.0 lb. hay and 20.0 lb. concentrates daily/cow. In the second period 2.0 lb. hay and 24.0 lb. concentrates were fed to each cow daily and in the third 2.0 lb. hay, 5.0 lb. delignified straw and 20 lb. concentrates. In the 4th—2.0 lb. hay and 20.0 lb. concentrates and in the 5th—18.0 lb. hay and 10.0 lb. concentrate daily/cow.

The milk fat content which averaged 3.5% in the 1st period fell to below 2.0% until the last period at which time it became normal. Blood lipids fell in period 2 but again rose to near normal when straw was fed. Blood glucose values did not change with rations. While on low hay, volatile acid production in the rumen came to a peak more quickly and the pH remained lower for a longer time than when the cows were fed more hay. The amount of acetate produced appeared to be reduced while the production of propionate remained relatively constant. Except while eating, the reticulo-rumen contracted more slowly while on the low-hay diet. The rumen contained less material when the low-hay diets were utilized and this appears to explain the loss of weight observed when animals were placed on low-hay diets. The digestion of starch appears to be

quite complete in the rumen on all rations. Time spent ruminating was considerably less when a low-hay ration was fed.

The low-fat content of milk from cows on the low-hay diet was explained on the basis that less acetate was formed and absorbed from the rumen than when normal rations were used.

J. D. Donker

176. Effect of treatment of dairy pastures with BHC and DDT on the flavour and composition of milk, cream, and butter. F. H. McDowall, M. R. Patchell, F. Hurst, and J. M. Kelsey. New Zealand J. of Sci. and Agr., **A37**, 2: 146. 1955.

Treatment of dry closely grazed pastures with 2 lb. of lindane or with 2 lb. of DDT per acre, with a 3-week interval before grazing, did not cause taint in milk, cream, or butter. The butter contained 4-6 p.p.m. of benzene hexachloride and no DDT. Treatment under similar conditions with mixed isomers of BHC caused a slight taint in the milk, cream, and butter, and the butter contained 16-18 p.p.m. of BHC.

Treatment of long pastures, when wet, and grazing of the area immediately gave (a) with lindane a "chemical taint" in the milk, cream, and butter; (b) with BHC (mixed isomers) a strong "earthy" or "musty" taint in all products; (c) with DDT a "sweetish" taint in the milk and cream but not in the butter. The "chemical" and "earthy" taints persisted in the milk for some time after the cows were removed from the treated area.

W. K. Jordan

177. Milk composition studies in the hormonal induction of lactation using identical twin dairy cattle. D. R. Perrin. New Zealand J. of Sci. and Technol., A37, 2: 88. 1955.

Changes in milk composition have been studied with identical twin heifers brought into lactation by treatment with progesterone and synthetic oestrogen. In general, the mammary secretions were initially colostral in nature, but, as the milk flow became established, gradually altered to a milk of normal composition.

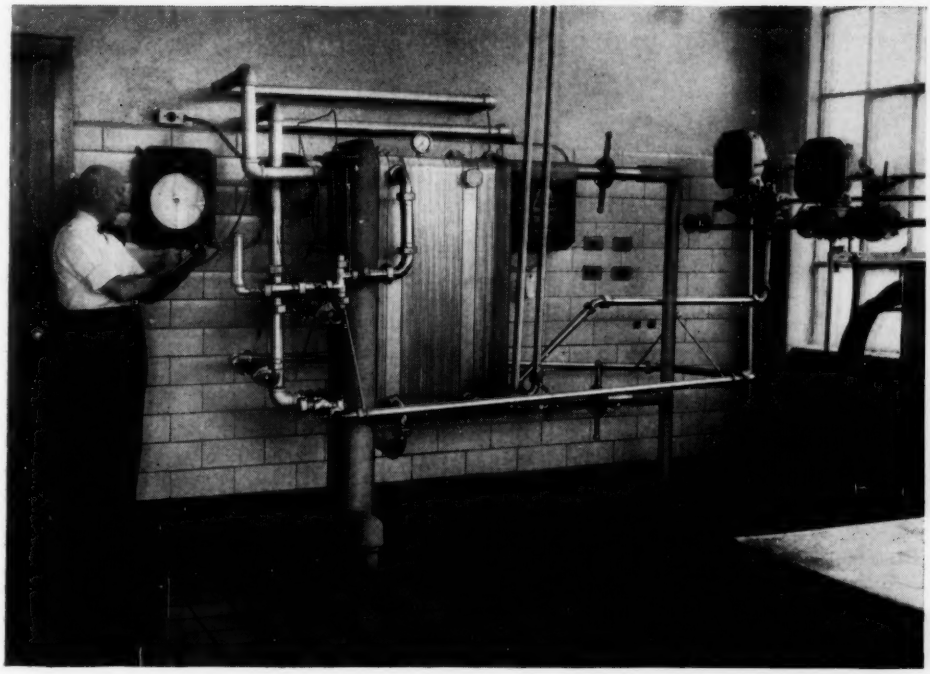
W. K. Jordan

SANITATION AND CLEANSING

178. C-I-P does a difficult job better. A. C. Fay, H. P. Hood & Sons, Boston, Mass. Milk Plant Monthly, **44**, 12: 35. 1955.

In-place cleaning of either glass or stainless steel affords a considerable saving in cleaning costs. The methods used at the H. P. Hood and Sons Boston plant are discussed. Daily sponging is used during the circulation of the detergent. The C.I.P. method serves its best purpose in long lines with relatively few side connections and valves, and does not lend itself to short lines with a valve every few feet. Gaskets on cold lines should be replaced once a year; on hot lines every three months.

C. J. Babcock



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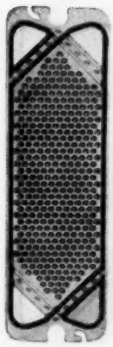

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